

EXPERIMENTAL SIALOGRAPHY

The Effects of Retrograde Infusion of Radiographic Contrast
Media on the Rat Submandibular Gland

Eva Qwarnström



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Media on the Rat Submandibular Gland

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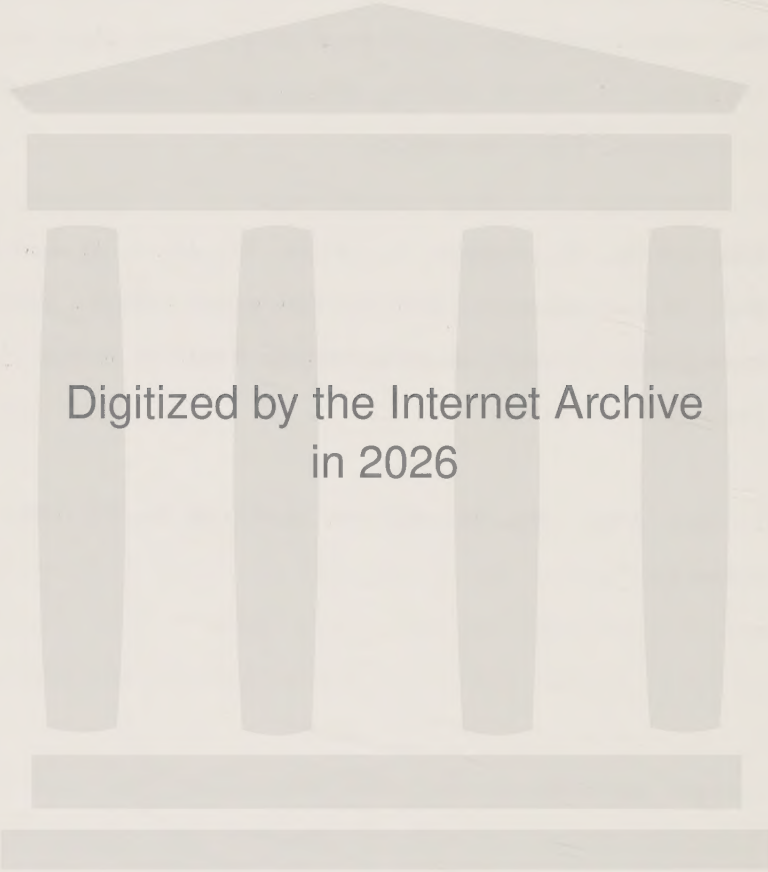
PREFACE

This thesis is based on the following papers:

- I. Qwarnström, E., Gjørstrup, P. and Omnell, K-Å. Sialography of the submandibular gland in the rat. An experimental model including measurement of intraglandular pressure. Dentomaxillofac. Radiol. 1981, 10:27-34.
- II. Qwarnström, E.E. and Hand, A.R. A light and electron microscopic study of the distribution and effects of water-soluble radiographic contrast medium after retrograde infusion into the rat submandibular gland. Archs. oral Biol. 1982, 27:117-127.
- III. Qwarnström, E.E. and Hand, A.R. A light and electron microscopic study of the effects of retrograde infusion of lipid-soluble radiographic contrast medium into the rat submandibular gland. Archs. oral Biol. 1982, 27:705-714.
- IV. Omnell, K-Å and Qwarnström, E.E. A technique for intraoral cannulation and infusion of the rat submandibular gland. Dentomaxillofac. Radiol. 1983, 12:13-15.

- V. Qwarnström, E.E., Omnell, K-Å and Hand, A.R. A morphologic study of the recovery of the rat submandibular gland after retrograde infusion. I. Water soluble radiographic contrast medium. J. Oral Pathol. 1983, 12:417-429.
- VI. Qwarnström, E.E. and Hand, A.R. 1983. A morphologic study of the recovery of the rat submandibular gland after retrograde infusion. II. Lipid soluble radiographic contrast medium. J. Oral Pathol. 1983, 12:430-445.
- VII. Qwarnström, E., Bodner, L., Baum, B., Hand, A. and Omnell, K-Å. Saliva secretion from the rat submandibular gland after retrograde infusion of radiographic contrast media. J. Dent. Res. 1984. In press.

In the text, the papers are referred to by their Roman numerals.



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INTRODUCTION

The need to evaluate the internal structure of salivary glands for diagnostic purposes was early recognized. Sialography, a radiographic examination that comprises filling of the luminal system with contrast medium, has been used for a long time in clinical diagnosis. Charpy (Poirier and Charpy, 1904) was the first to demonstrate a salivary gland duct system in a radiograph, after infusion of mercury. The first sialogram of a live patient, using bismuth as a contrast agent, demonstrated a sialolith in the main excretory duct of the submandibular gland (Arcelin, 1913). The examination was first used on a practical diagnostic basis in the mid-1920's (Barsony, 1925; Uslenghi, 1925; Carlsten, 1926).

A number of other methods, such as scintigraphy (isotope scanning) (Gates and Work, 1967; Enfors et al., 1969; Schall, 1971; Parret and Peyrin, 1979) and ultrasound examination (Neiman et al., 1976; Bruneton et al., 1983), have been used in diagnosis of salivary gland diseases. Scintigraphy makes possible evaluation of both the morphological and functional status of the gland (Harden, et al., 1967; Sorsdahl et al., 1969; Fiori-Ratti et al., 1977). It is used for evaluation of the parenchyma, primarily for detection of defined lesions in salivary glands (Gates, 1972). In addition, alterations in the minor salivary glands are detectable by scintigraphy. Comparative studies on the diagnostic value of sialography and scintigraphy have shown that they are often



complementary (Gates, 1977; Tainmont et al., 1979; Lindvall, 1980; Cogan and Gill, 1981). The two examinations are sometimes used together in diagnosis of parotid and submandibular lesions. Ultrasound is useful in differentiating between cystic and solid lesions (Neiman et al., 1976) and in distinguishing between benign and malignant tumors (Bruneton et al., 1983). Recently, computerized tomography alone (Bryan et al., 1982), or more frequently together with filling of the duct system with contrast medium (Mancuso et al., 1979; Rice et al., 1980; Carter et al., 1981 a, b, Som et al., 1981; Sone et al., 1982), has been used in salivary gland diagnosis. This examination also allows evaluation of the surrounding soft tissues and the relationship of the lesion to the facial nerve. For evaluation of the function of the gland sialochemistry, sialometry (Mandel, 1980) and radiosialometry (Kumlien and Wiberg, 1974) have been employed.

Conventional sialography gives a detailed view of the duct system and an accurate estimate of the size of the gland (Ericson and Hedin, 1970; Ericson, 1970). It offers the possibility of diagnosing a wide variety of pathological alterations, including both defined and diffuse lesions. A good evaluation of the location of the lesion as well as possible involvement of the adjacent hard tissue, can be obtained. In radiographs taken at various times following the examination, the rate of removal of the contrast medium can be determined. This procedure, first introduced as secretory sialography (Rubin et al., 1955), provides information about the

function of the gland (Park and Mason, 1966; Blair, 1976). For these reasons, sialography is still a widely used diagnostic tool (Blair, 1976; Lowman and Cheng, 1976; Adam et al., 1983).

The value of the sialographic examination is, to a great extent, dependent on the degree of filling of the gland. Opinions vary as to what is the optimal filling from a diagnostic standpoint. With ductal filling, where the contrast medium is confined to the lumina of the intralobular ducts (Ericson, 1968; 1973), diffuse radiopacity of the gland is avoided. At parenchymal filling, the contrast medium is thought to be present also in the acinar lumina (Ollerenshaw and Rose, 1951; Park and Mason, 1966; Lowman and Cheng, 1976). Further infusion eventually results in total ductal and glandular distension (Osmer and Pleasants, 1966).

Both lipid- and water-based compounds have been used as contrast media. Lipid-based substances were introduced in the beginning of the 1920's (Sicard and Forestier, 1921) and were soon thereafter used in sialography (Carlsten, 1926). It was eventually found that these iodized oils could cause a granulomatous reaction (Epsteen and Bendix, 1954). Although it was noted that some oil derivatives are less harmful than others (Epsteen and Bendix, 1954) none of them meet the requirements for an ideal contrast medium (Neustaedter, et al., 1933; Nitsche and Valyi, 1962). Water-soluble media, primarily developed for urography (Cameron, 1918; Dennis, 1956), have more recently been used routinely in sialography (Gullmo

and Book-Hederstrom, 1958). Most investigators now use water-soluble media (Drevattne and Stiris, 1964; Blair, 1973) but lipid-based media are still employed (Lowman and Cheng, 1976).

The contrast medium is administered via the main excretory duct, originally by hand injection technique using a glass cannula (Payne, 1931). In order to improve the technique for the sialographic procedure and further enhance its diagnostic value, new methods for performing the examination have been proposed. Thus, various types of cannulas have been designed and alternative ways of cannulating the main excretory duct have been suggested (Thomas, 1956; Liverud, 1959; Park and Bahn, 1968; Rabinov and Joffe, 1969; Lebowitz and Laskin, 1978; Rabinov, 1981; Som and Khilnani, 1982). A commonly used indicator of filling of the gland is pain felt by the patient, introduced by Payne (1931). In addition, injection of a specific volume has been recommended (Winsten et al., 1956). New methods have dealt with alternative ways for administering the contrast medium in order to get optimal filling of the gland. Thus filling by pressured gas (Ollerenshaw and Rose, 1951) or by prepumped pressure, i.e. isobaric sialography (Sazama, 1973), have been suggested. With the use of water-soluble contrast media, hydrostatic sialography (Gullmo and Book-Hederstrom, 1958; Park and Mason, 1966; Park and Bahn, 1968; Blair, 1973) has evolved. In order not to have to rely on the patients subjective feeling of pain, continuous observation of the filling phase using an image intensifier system has been done, and spot radiographs taken

throughout (Feldman, 1965; Berg, 1969; Yune and Klatte, 1972; Beideman and Tatoian, 1977; Kushner and Weber, 1978). The pressure developing during infusion (Ferguson et al., 1977) as well as the pressure in the gland after stop of infusion (Zijlstra and Ten Bosch, 1975) have been used to determine the optimal filling of the gland.

Whereas numerous techniques have been developed for the purpose of improving the diagnostic value of the examination, few studies have dealt primarily with the effect of the sialographic procedure on the glandular tissue (Ranger, 1956). It has been suggested however, that prolonged injection may be harmful to the salivary gland parenchyma (Rubin et al., 1955; Garusi, 1964) and that sialiectasis seen in Sjogrens syndrome could be due to distortion of weakened duct walls during infusion of lipid-based contrast media (Bloch et al., 1965). Experimentally, it has been shown that the pressure induced in the luminal system during sialography can cause damage to the parenchymal tissue, resulting in alteration in the sialographic appearance (Garrett et al., 1976).

To be able to systematically study the effect of retrograde infusion on salivary gland structure and function, an experimental model for sialography was developed using the rat submandibular gland. With an animal model, a large number of examinations of normal glands could be carried out. This allowed standardization of basic parameters since no pathological alterations or functional disturbances had to be taken into consideration. It also made

possible the use of each contralateral gland as a control. Further, the alteration in the entire gland could be evaluated, rather than a small portion obtained from a biopsy. The rat submandibular gland was chosen since both its morphology (Scott and Pease, 1959; Tamarin and Sreebny, 1965; Young and van Lennep, 1978) and several aspects of its physiology (Thulin, 1974, 1976) are well documented. In addition, conducting the radiographic examination, the gland can easily be projected free from the bones of the skull and the vertebral column, in a frontal projection.

The model was based on a correlation between different degrees of filling of the gland as evaluated radiographically and the pressure developing during continuous infusion (I). Pressure monitoring has previously been suggested as an aid in clinical sialography in order to determine the optimal filling of the gland (Zijlastra and Ten Bosch, 1975; Ferguson et al., 1977). The present system correlates various degrees of filling based on the appearances of the gland in the sialogram with specific parts of the pressure curve obtained by continuous measurement during infusion. The experimental model was used to investigate fundamental aspects of sialography.

The system has been used for retrograde infusion of both water- and lipid-soluble contrast medium as well as isotonic saline solution. The effects of retrograde infusion of these media on the morphology of the gland have been examined by light and electron

microscopy, after various degrees of filling of the gland. The immediate alterations (II, III) as well as the recovery of the gland, and changes occurring at later times after filling (V, VI) were studied. In addition, the distribution and fate of the infused contrast media and the isotonic saline were determined (II, III). For the water-based medium and the saline solution (II) this was done directly by the use of a soluble phase tracer, horseradish peroxidase (HRP), dissolved in the media. The function of the gland after retrograde infusion with the two contrast media was evaluated (VII). For these studies, stimulated saliva was collected at different times after filling of the gland, and flow rate and saliva composition were determined.

AIMS

1. To design an experimental model for sialography using the rat submandibular gland, permitting controlled studies on the sialographic procedure.
2. To investigate the morphological alterations induced by retrograde infusion of water- or lipid-soluble radiographic contrast medium and to compare the effects of different degrees of filling of the gland.
3. To determine the distribution and fate of the water- and lipid-soluble contrast media in the glandular tissue after retrograde administration.
4. To design a technique for intraoral cannulation of the rat submandibular gland allowing high enough pressure to make possible long term studies of the sialographic procedure.
5. To evaluate the recovery of the glandular tissue after retrograde infusion and to determine eventual morphological alterations occurring at later times following filling of the gland with water- or lipid-soluble radiographic contrast medium. In addition, to study the significance of the degree of filling with these media with respect to the long term effects.

6. To obtain information about the function of the gland at various times after different degrees of filling with water- or lipid-soluble radiographic contrast medium.

MATERIAL AND METHODS

RADIOGRAPHIC STUDY

The experimental model for sialography was based on a correlation between the pressure developing during infusion and the degree of glandular filling. Twenty-four submandibular glands from 16 Wistar rats were used. The main excretory duct was cannulated extraorally (Ohlin, 1965) in anesthetized animals (chloral hydrate, 240 mg/kg b.w., i.p.) and secretion was stimulated (methacholine, 2-5 μ g/kg b.w., i.v.) 10 min. before infusion. Water-soluble (Urografin 60%, iodine content 292 mg/ml, viscosity 4 mPa.s at 36°C) or lipid-soluble (Lipiodol UF, iodine content 480 mg/ml, viscosity 25 mPa.s at 36°C) contrast medium was infused into the gland at a constant rate of 0.008 ml/min using a syringe pump. This was the lowest rate with which a complete filling of the duct system could be obtained using the water-soluble medium. The developing pressure was monitored throughout the experiment using a displacement transducer connected to an amplifier and a recorder, similar to the system described by Emmelin et al., 1968. Calibration was done with a pressure bottle and a mercury manometer. Radiographs (non-screen film) were taken at thirty second intervals throughout the experiment using a Lysholm precision apparatus (rotating anode 0.6 x 0.6 mm). A narrowed beam (40 mm; 55 kV; 120 mAs) was angled medially and anteriorly in order to free project the gland from the bones of the

skull. As a comparison, the pressure developing during infusion of isotonic saline solution (0.9%) was recorded.

The pressure development recorded during infusion was characterized for each of the three media. For the two contrast media, various degrees of filling of the gland, seen in the sialogram, were described. Finally, a comparison was made between different parts of the obtained pressure curves and the degrees of filling of the gland.

MORPHOLOGICAL STUDIES

Immediate effects:

The experimental model was used to investigate the immediate effects on the morphology of the glandular tissue of retrograde infusion of water-soluble (Renografin-60, iodine content 288 mg/ml, viscosity 4 mPa.s at 36°C, USA equivalent to Urografin 60%) and lipid-soluble (Lipiodol UF, the same as used in the radiographic study) radiographic contrast media. The left submandibular glands of 34 anesthetized (chloral hydrate, 400 mg/kg b.w., i.p.) adult Sprague-Dawley rats were cannulated extraorally. Contrast medium or isotonic saline solution (0.9%) was infused retrogradely, without prior stimulation, at a constant rate of 0.008 ml/min using a syringe pump. Filling of the gland was done during continuous measurement of the intraglandular pressure using a displacement transducer and a recording device similar to that employed in the radiographic

study. Infusion was stopped at different parts of the pressure curve corresponding to different degrees of filling, and the glands immediately fixed by vascular perfusion with a modified Karnovsky's (1965) fixative (2% glutaraldehyde, 2% formaldehyde in 0.1 M cacodylate buffer with 0.05% CaCl₂, pH 7.4). Both submandibular glands, the left experimental and the contralateral that served as a control, were removed and the glandular tissue prepared for light and electron microscopy. Postfixation in 1% osmium tetroxide in sucrose buffer (0.1 M cacodylate buffer with 7% sucrose added, pH 7.4) and block staining in uranyl acetate (0.5% in distilled water with 5% sucrose) preceded dehydration in a graded series of ethanols and embedding in Spurr's (1969) resin.

For microscopic visualization, a soluble phase tracer, horseradish peroxidase (HRP, 10 mg/ml), was dissolved in the water-soluble medium and the isotonic saline solution prior to infusion. Following fixation, tissue from these glands was chopped into 75 μ m sections and incubated in a medium containing diaminobenzidine (1 mg/ml) and 0.02% hydrogen peroxide (pH 7.4) (Fahimi, 1970) prior to postfixation and processing for light and electron microscopy as described above.

Thick (1 μ m) sections, unstained or stained with toluidine blue, were examined by light microscopy. Thin sections of selected areas were cut with a diamond knife and mounted on bare copper grids. The sections were stained with uranyl acetate and lead citrate and examined in a Zeiss EM-10A electron microscope. In addition, a thin transverse slice from the center of each gland was

processed, embedded in JB-4 plastic and sectioned. The sections were left unstained, stained with light green counterstain, haematoxylin-eosin, or periodic acid-Schiff-haematoxylin before light microscopic examination.

Morphological alterations present in the various segments of the intraglandular duct system and acini were described. The severity of the changes at various degrees of filling were evaluated for both contrast media and isotonic saline solution. Further, the distribution and fate of the infused media was determined. This was done directly for the water-based compound and the isotonic saline solution by localization of the HRP reaction product. Since a suitable tracer could not be found for the lipid-based material, its localization was assessed by inference.

Recovery and long term effects:

Studies on the recovery of the gland following the sialographic procedure were carried out using the same experimental model. The main excretory duct of the left submandibular gland of 60 anesthetized (sodium pentobarbital, 45 mg/kg b.w. i.p.) adult Sprague-Dawley rats was cannulated intraorally through its natural orifice, located on the sublingual caruncle. The tongue was pulled upwards and aside providing access to the orifices of the ducts. A polyethylene tubing was inserted after dilation of the outer portion of the duct. The soft tissue lining the papilla was allowed to dry and adhere to the tubing. To tolerate high pressures, a cotton

ligature was tied around the stretched papilla. The left glands were infused with water-soluble (Renografin-60) or lipid-soluble (Lipiodol UF) radiographic contrast medium at a rate of 0.008 ml/min during monitoring of the intraglandular pressure as previously described. The right gland in each animal served as control. The experimental glands were subjected to ductal and slight parenchymal filling or infused until heavy parenchymal filling was achieved, as determined by the development of the recorded pressure. These two degrees of filling correspond to the beginning of phase B and the middle of phase C, respectively, in the radiographic study (1).

Animals subjected to different degrees of filling with either medium were killed at various times after the procedure (10, 20, 30 h; 4, 7, 10d). The submandibular glands were fixed by vascular perfusion, the tissue prepared for light and electron microscopy and examined as previously described. Further, sections from a transverse slice of glandular tissue, embedded in JB-4 plastic were stained with Giemsa.

The pattern of recovery of the glandular tissue was examined microscopically. Additional changes occurring in the tissue at later times following the procedure were studied. The results allowed a comparison between the long term effect of infusion of the two types of contrast media. In addition, an evaluation of the impact of the degree of filling on the recovery of the gland was made.

FUNCTIONAL STUDY

For studies concerning the function of the gland, stimulated saliva (pilocarpine-HCl 2.5 mg/kg b.w s.c) was collected at different times (immediately, 20 h, 1 wk and in some cases 2 wk) following cannulation of the main excretory duct and infusion of water- (Renografin-60) or lipid-soluble (Lipiodol UF) contrast medium. Left submandibular glands of 32 Sprague-Dawley rats were filled retrogradely (0.008 ml/min) during continuous pressure monitoring, while the contralateral gland served as a control. The same two degrees of glandular filling chosen for the long term morphology studies (ductal and slight parenchymal filling and heavy parenchymal filling) were selected, as determined by the development of the intraglandular pressure.

Secretion was analysed for flow rate, and concentrations of protein, lactoperoxidase, Na^+ and K^+ . Flow rate was determined gravimetrically, assuming a specific gravity of saliva equal to 1. Total protein was determined by absorbance at 215 nm (Arneberg, 1971) and Na^+ and K^+ concentrations by atomic absorption spectrometry. Lactoperoxidase activity was determined by measuring oxidation of iodine in the presence of hydrogen peroxide at 350 nm (Morrison, 1970) and albumin content by immunosorbent assay (ELISA) (Baum et al., 1982). Data were expressed as ratio values of the infused gland/control gland for each animal and compared to ratios from rats where neither gland was infused with contrast medium.

RESULTS

RADIOGRAPHIC STUDY

Retrograde infusion of water- and lipid-soluble media into the rat submandibular gland resulted in reproducible and, for each medium, characteristic pressure development. The intraglandular pressure recorded during infusion of the water-soluble material into the salivary gland reached two major peaks at about 40 mmHg and eventually decreased to a plateau at about 30 mmHg (Fig. 1a). The maximum pressure and the plateau level were in the same range as those obtained during administration of isotonic saline solution, although the latter resulted in only one major peak (Fig. 1b). Infusion of the lipid-based medium caused a much higher intraglandular pressure. It resulted in a stepwise increase that, using the Wistar rats, reached 250-300 mmHg (Fig. 1c). Common to all types of pressure curves was an initial short interval after start of infusion before the pressure started to rise.

In order to determine to what extent the recorded pressure reflected events in the gland, the properties of the infusion system alone were studied. Flow of the water- and lipid-soluble contrast media through the system alone, i.e. when the glass cannula was open to the air, caused a pressure increase to a plateau, at 6 and 18 mmHg, respectively (Fig. 2). Infusion of isotonic saline solution did not result in any measurable increase in pressure. Compliance during infusion of water-soluble medium into the gland was at least

(0.20 $\mu\text{l/mmHg}$) about twice that noted during infusion into the system after closing the tubing at the level of the main excretory duct of the gland (0.12 $\mu\text{l/mmHg}$). Using the lipid-based medium, the compliance in the steepest part of the initial increase was about the same as that in the closed system (0.08 $\mu\text{l/mmHg}$). Whereas in other parts of the curve, it was noticeably higher.

For both contrast media, it was found that the radiographic appearances of the gland during filling with contrast medium were analogous to those that can be observed in clinical sialography. Four consecutive stages of filling were distinguished, based on specific appearances of the gland (1, Figs. 3, 5). Ductal filling (A) was followed by parenchymal filling (B), the two degrees of filling most commonly used in clinical sialography. Continued infusion resulted in the appearance of larger single dots of contrast medium, heavy parenchymal filling (C), which eventually led to total confluence of contrast medium and opacification of the gland (D), corresponding to two degrees of overfilling. These stages could be recognized for both water- and lipid-soluble radiographic contrast media although the appearance of the gland was affected by the type of the infused material. Infusion with water-based medium eventually resulted in a diffuse opacification, whereas glands infused with lipid-based medium revealed their contours also at later stages of filling. A more distinct outline of the glandular structure was consistently seen following infusion of the lipid-based compound. A successive filling of the gland as described, resulted in the

characteristic pressure development for the medium used. The different appearances of the gland, seen in the sialogram during infusion, could be correlated with specific parts of the corresponding pressure curve. Except for complete outlining of the duct system (A), the various stages of filling of the gland occurred at shorter infusion times using the lipid-based material. In addition, the recorded pressure was higher at every given stage, than at the comparable degree of filling during infusion of the water-based medium.

Variations in the height of the pressure peaks, as well as in the relation of different parts of the curve to the time axis, were observed. However, the overall shape of the curves describing the development of the pressure during proper infusion was highly reproducible. A lesser degree of filling of a lobe or part of a lobe, occasionally seen in the sialogram during infusion of the water based medium, did not alter the general development of the recorded pressure. However, rapid changes in the pressure development were noted in cases where the filling of the gland was generally incomplete and infusion into the main excretory duct was affected due to leakage or blockage in other parts of the system.

MORPHOLOGICAL STUDIES

The appearances of the pressure curves obtained during infusion of the water-based medium and isotonic saline solution were

largely the same as described above. In most of these studies however, a sharp small peak of at most 10 mmHg occurred during the first pressure increase. Subsequent experiments showed that prior stimulation with methacholine resulted in an even first pressure increase, like that obtained previously. The pressure development obtained during infusion of the lipid-based compound was initially the same as previously described, both regarding time and pressure recorded at the various plateaus. After reaching about 150 mmHg, a decrease to a plateau followed, instead of an increase as previously noted. This was at a part of the curve comparable to the last stage of filling included. Subsequent experiments suggested that the observed difference was due to the use of different strains of rats in the two studies.

Immediate effects:

The morphological alterations caused by retrograde infusion of water-soluble radiographic contrast medium consisted primarily of dilation of the intralobular ducts. All three components of the intralobular duct system, striated, granular and intercalated ducts, were affected, each segment showing a characteristic appearance (II, Fig. 4). The lumen of the granular and striated ducts presented a scalloped appearance, with the junctional regions projecting into the lumen (II, Fig. 7). The lumen of the intercalated ducts became irregularly enlarged and considerable flattening of the duct cells occurred (II, Fig. 6). These alterations successively became more

pronounced as infusion continued, and a larger proportion of the tissue was affected at later stages of filling. The intercalated duct was most severely altered and widening of the desmosomes between these duct cells eventually occurred (II, Fig. 6 inset). The main excretory duct was apparently unaffected by the procedure.

The initial alterations in the duct system were seen at short infusion times, before the tracer, dissolved in the contrast medium, was present in the tissue. Electron dense reaction product first appeared in the tissue in glands where infusion was continued until the latter part of the first pressure increase was reached. It was first detected in the lumina of the ducts and acini and then seen in the intercellular spaces between the different parenchymal cells (II, Figs. 5 inset; 8 a, b). Penetration of the tracer through the junctional complexes was most apparent in the acini. In the intralobular ducts, the presence of the tracer in the intercellular spaces, was most frequently associated with luminal dilation. It was also occasionally seen in membrane bound vesicles in the duct cells (II, Fig. 9 inset). Eventually, the tracer was present at the basal surface of the cells (II, Figs. 8 b, 9) and subsequently filled the surrounding connective tissue. At longer infusion times, the reaction product was found between the endothelial cells and in the lumina of the blood and lymphatic vessels (II, Figs. 10, 11).

The type of morphological changes induced by infusion of isotonic saline solution were the same as those occurring during

administration of water-soluble radiographic contrast medium, but were generally less pronounced. The distribution and fate of the saline were, by and large, the same as described for Renografin. In addition, the apical membrane of the parenchymal cells became heavily stained with reaction product and some duct cells showed the presence of tracer in the cytoplasm (II, Figs. 12-14).

The earliest morphological changes occurring during infusion of the lipid-based contrast medium were dilation of the intralobular ducts and subsequent widening of the desmosomes, the same as described for the water-soluble medium (III, Figs. 3-5). As infusion continued, additional morphological alterations were seen in the glandular tissue. Due to continuous widening of the ductal lumina and stretching of the apical surface, the scalloped appearance of the granular and striated ducts successively became less apparent (III, Fig. 6). In addition, the microvilli on the apical membrane of the duct cells were bent towards the luminal surface of the cells. Infusion of the lipid-based medium eventually resulted in formation of cytoplasmic vacuoles, some of which were seen to be in continuity with the lumen (III, Fig. 7). Changes in the acini comprised dilation of the acinar lumina and widening of the intercellular canaliculi (III, Fig. 8). As infusion continued these changes successively became more severe, particularly in the intercalated duct segment and the acini (III, Fig. 11). Pronounced deformation of the cells in these segments occurred, resulting in compression of the cellular organelles. The dilated lumina of the ducts and acini and

the forming vacuoles were frequently only partly filled with secretory product (III, Fig. 9). The secretory material in each luminal profile was separated from an apparently empty area by an electron dense interface.

Light microscopic studies of transverse slices of the whole gland revealed an uneven distribution of both types of contrast media and the saline solution. This was demonstrated directly for the water-soluble contrast medium and isotonic saline solution by the use of horseradish peroxidase (II, Fig. 5). Thus, areas containing large amounts of tracer were present next to areas where no reaction product could be seen. After longer infusion times a larger proportion of the tissue contained HRP reaction product. In addition, the severity of the morphological alterations varied in different parts of the gland, correlating with the distribution of tracer. In glands infused with the lipid-based medium, variations in morphological appearances were seen throughout the gland (III, Fig. 12). Thus, in some areas, the tissue had an almost normal appearance whereas in others, severe alterations were seen in all parenchymal components. This was apparent at all stages of filling. As infusion continued, a greater proportion of the tissue showed morphological alterations.

Recovery and long term effects:

The alterations seen in the tissue at the earliest time (10 h) after infusion of water-soluble medium were similar to those observed

immediately following the procedure. They consisted mainly of dilation of the intralobular ducts, with an irregular and scalloped appearance of the luminal border (V, Fig. 3). The severity of the morphological alterations varied to some extent between glands subjected to the same degree of filling, and also between different parts of the same gland. In general, the changes were more frequent and more pronounced in glands subjected to heavy parenchymal filling than in those where infusion was stopped when ductal filling was achieved. Pronounced widening of the lumina, most frequently seen in the overfilled glands, resulted in the presence of apparent vacuoles in the apical cytoplasm of the intercalated duct cells (V, Fig. 4). The desmosomes were occasionally widened at this time. In the granular duct segment, apparent vacuole formation and compression of the secretory granules were frequently observed (V, Fig. 5), particularly in the overfilled glands. After ductal filling, the size of the luminal diameter had returned to normal at 20 h, although the irregularity of the luminal border could still be observed at 30 h after infusion. In glands subjected to heavy parenchymal filling, both the dilation of the intralobular ducts and the irregularity of the luminal border were present at 30 h.

Alterations in the duct system following infusion of the lipid-based medium were the same as those described for the water-based medium but much more pronounced and also lasted longer. Dilation of the intralobular ducts was much more severe. In addition, large vacuoles were present in the various duct segments,

particularly in the striated ducts (VI, Figs. 2, 3). They were most frequently seen in the overfilled glands and quite often contained electron dense material. The vacuoles were usually localized in the cytoplasm of the duct cells, but occasionally, they were contained within macrophages present in the intercellular spaces of the ducts.

Alterations in the acini were seen throughout the recovery period in glands subjected to heavy parenchymal filling with the water-based medium and in both groups of animals following infusion of the lipid-based compound. The alterations comprised dilation of the acinar lumina and widening of the intercellular canaliculi, resulting in a star shaped appearance of the lumina, the same as that noted immediately after infusion of the lipid-based compound (III, Fig. 8). This was most prominent at the acinar-intercalated duct junction. Confluence of secretory granules was frequently seen (V, Fig. 6), particularly after infusion of the lipid-based compound. In both groups of overfilled glands, the confluence of secretory granules resulted in the formation of cytoplasmic vacuoles (VI, Fig. 4). Accumulation of secretory granules in the cytoplasm was pronounced in some acinar cells in glands subjected to heavy parenchymal filling with the lipid-soluble medium (VI, Fig. 5).

Lysosomes and autophagic vacuoles were present in the parenchymal cells at early times following infusion with the lipid-based medium, most frequently in the acinar cells of the

overfilled glands (VI, Fig. 6). Infusion of the lipid-based compound resulted in widening of the intercellular spaces, particularly in the acini and intercalated ducts (VI, Figs. 7, 11). In addition, displacement and folding of the basement membrane occurred in the overfilled glands.

An inflammatory infiltrate which reached a maximum at 20 h was seen in the glandular tissue after both degrees of filling with either medium (V, Fig. 8). It was more prominent in the overfilled glands, and also lasted longer. Initially, it consisted of polymorphonuclear leukocytes and macrophages; whereas at later times, an increase in mast cells and plasma cells was seen. In addition, an infiltration of eosinophils occurred. The reaction was stronger in glands infused with the lipid-soluble medium. Whereas the infiltration in glands subjected to ductal filling was similar to that noted after infusion of the water-based medium, the reaction in the overfilled glands was, in several aspects, quite different. At early times, cyst-like spaces, surrounded by a large number of inflammatory cells, were seen in the interlobular connective tissue (VI, Fig. 8). In addition, inflammatory cells were quite often present inside the basement membrane of the different parenchymal components (VI, Fig. 9). Macrophages frequently contained vacuoles with secretory material or apparent contrast medium. A large number of macrophages enclosing rounded spaces in the parenchyma was commonly seen at later times (VI, Figs. 10, 11). The spaces frequently contained electron dense material.

At 30 h after ductal filling with the water-based medium, the inflammatory infiltrate was reduced, and generally, no alterations were seen in the glandular tissue. Following infusion with the lipid-soluble medium, the inflammatory infiltrate was still present at 4 d although markedly reduced, and the tissue generally had a normal appearance at this time. In glands subjected to heavy parenchymal filling, the inflammatory infiltrate was still prominent at 4 d after the procedure and marked morphological alterations were present in both groups of animals. Alterations were also seen at 7 d in these glands. Variations in the severity of the changes were striking both between glands, and between different areas of the same gland. The parenchyma in some overfilled glands had a normal appearance whereas in others, degenerative changes were seen in ducts and acini.

These atrophic changes mainly consisted of a decrease in size of the parenchymal cells, with depletion of the secretory granules in acinar and granular duct cells (V, Figs. 9, 10). In addition, an increase in the connective tissue stroma and in the number of small blood vessels was apparent. In glands infused with the lipid-based medium these changes were generally much more pronounced (VI, Figs. 13, 14). Cytoplasmic vacuoles, occasionally containing electron dense material, were present in the acini (VI, Fig. 11), leading to deformation of the acinar cells. Eventually, macrophages were occasionally seen to surround groups of deformed parenchymal cells. The tissue of the overfilled glands, examined 10 d after infusion

with either medium, generally had a normal appearance; although in some areas atrophic alterations of the parenchyma were still observed.

The main excretory duct had an appearance similar to the control at all times following either degree of filling, with water- or lipid-based medium. A slight increase in luminal diameter was occasionally seen in the interlobular ducts of the overfilled glands.

FUNCTIONAL STUDY

In glands subjected to ductal filling no alterations were detected in flow rate (VII, Tables 2, 3). A significant reduction occurred at 20 h after heavy parenchymal filling with either medium. The flow rate was also reduced immediately after retrograde filling with the lipid-based medium, but this was not statistically significant. By 1 wk the values were comparable to controls.

The composition of saliva was not markedly affected by retrograde infusion. In glands subjected to ductal filling no changes occurred, and only a few significant alterations were noted following heavy parenchymal filling. Thus, the protein concentration was increased immediately following heavy parenchymal filling with water-soluble medium, but had returned to normal by 20 h. At this time, an increase in protein concentration was noted in saliva from

glands subjected to heavy parenchymal filling with the lipid-soluble medium, but was not statistically significant.

A high lactoperoxidase activity was observed in saliva collected at 1 wk after heavy parenchymal filling with either medium, although this was not significantly different from controls. The only change in electrolyte concentrations was a small but significant increase in K^+ at 20 h following infusion of lipid-soluble medium, which had returned to normal at 1 wk. No significant changes in albumin concentration were noted at any time.

DISCUSSION

The basic structure of all major salivary glands is the same. Endpieces present in the lobules of the glands are made up of acinar cells. Successively larger ducts lead from the acini to the main excretory duct which empties into the oral cavity. Primary saliva consists of proteins, synthesized and released from the acinar cells by exocytosis, and water and electrolytes which enter at the level of the acini and intercalated ducts. The saliva composition is modified during transport through the duct system, before the final product reaches the orifices and is secreted into the mouth.

Sialography is a radiographic examination of salivary glands which comprises introduction of contrast medium into the luminal system via the main excretory duct. The studies described in the preceding section use the rat submandibular gland as an experimental model, for determining the effects of retrograde infusion on gland morphology and function. The system was based on a correlation between the appearance of the gland in the sialogram at different times during continuous infusion, and specific phases of the pressure curve obtained by measurement throughout the experiment. The developing pressure was used to estimate the degree of filling of the gland with water- and lipid-based contrast media. In addition, a correct infusion could be ascertained by using the system, since obstruction or leakage in the tubing or monitoring equipment resulted in rapid changes in pressure development.

The relation of the recorded pressure to the radiographic appearance and the morphological alterations will be discussed. Further, the morphological changes induced during infusion, and those occurring during the recovery period will be considered. The few functional alterations observed will be mentioned in relation to the morphological changes. Finally, the clinical relevance of the findings will be considered.

The variations in pressure development observed during infusion apparently reflected a changing distribution of contrast media observed in the sialograms. Using the water-based medium, a successive filling of the glandular ducts was noted during the first increase. The following pressure drop could be a result of a partly extraluminal distribution, suggested by the appearance in the second stage, parenchymal filling. The stepwise increase in the pressure noted during infusion of the lipid-based medium corresponds well to successively larger areas of contrast medium seen in the sialogram.

The overall shape of the pressure curves obtained with each medium in all studies were, with one exception, similar. Further, the various phases and their relation to time in the corresponding pressure curves generally agreed, using the same rate of infusion. This strongly suggests that the sialographic appearance of glands used in the morphological and functional studies would have progressed through the same sequence as described in the radiographic study (I, Figs. 3, 5). The sialographic appearance in

the last stage of filling with Lipiodol might have differed, however, as indicated by the different pressure development, observed with the Sprague-Dawley rats.

The recorded pressure reflects the resistance in the whole system. Initial dissimilarities in the characteristic pressure curves, obtained during infusion of the two contrast media and isotonic saline solution, are to a great extent due to differences in tubing and monitoring parts, since only saliva and saline are present in the gland. However, the initial short period before the pressure starts to rise may, to a great extent, be due to complete filling of the glandular lumen, since the pressure build up is immediate when the system is closed between the tubing and the gland (Fig. 1). In addition, it could reflect that the ductal volume of the resting gland, can be somewhat increased without resulting in a registered increase in pressure (Emmelin and Gjorstrup, 1974). The differences in the shape of the first pressure increase noted between stimulated and nonstimulated glands during infusion of the water-based medium and saline solution also indicate that events occurring in the gland are of importance.

Variations in pressure after complete ductal filling with either medium are primarily due to alterations in the glandular tissue, since the compliance in the system is comparatively low (Oberg, 1983). The coinciding of the first pressure fall during infusion of the water-based compound, containing HRP, with the leakage of the tracer into the intercellular spaces, strongly supports this view.

With the lipid-based compound, the frequent occurrence of widened acinar lumina at the first plateau, and the formation of vacuoles at the major peak also suggest that the pressure development, to a great extent, reflects morphological events.

Small differences in the recorded pressure, and its dependence on time, noted between various curves in all studies, may partly be due to differences in tension of the glands or amount of liquid present in the luminal system prior to infusion.

The uneven distribution of the infused media, observed at every degree of filling, presumably reflects an uneven distribution of pressure and resistance in the gland. This could partly be due to differences in length and width of the luminal system. Differences in the degree of filling of various parts of the gland, occasionally observed in the sialogram at later stages using Renografin, may be caused by changes in relative pressure during infusion. Resulting alterations in flow were easily seen because of the rapid resorption of the water soluble medium. Using the lipid-based compound these changes were less apparant radiographically, due to retention in the tissue.

Microscopically, the uneven distribution was demonstrated directly for water-soluble medium and isotonic saline. Irregular distribution of tracers following ductal filling has been noted previously in salivary glands (Garrett and Parsons, 1976; Parsons and Garrett, 1977) and in the exocrine pancreas (Pirola and Davis, 1970; Bockman et al., 1971), and probably reflects differences in

pressure and resistance in various parts of the glands. This is most likely influenced by differences in leakiness of the junctional complexes between cells in different areas. Variations in tracer permeability between acinar cells have been observed in the dog submandibular gland following arterial administration (Garrett et al., 1981) and could be a result of physiological activity of the cells (Junqueira et al., 1965; Parsons et al., 1977; Oliver and Hand, 1978; Garret et al., 1982; Mazariegos et al., 1984; Mazariegos and Hand, 1984). The variations in the severity of the morphological alterations, noted throughout infusion are presumably a direct result of such phenomena. Morphological data obtained during infusion of Lipiodol strongly suggest a similar uneven distribution of this medium.

Due to these variations, the development of the recorded pressure could not be directly correlated with specific morphological changes for any of the infused media, but reflects all events occurring in the tissue at a particular time. The overall pressure development in the gland, recorded after cannulation of the main excretory duct, most likely represents superposition of many pressure changes occurring in different parts of the gland. The characteristic and reproducible pressure development seen with each medium, is consistent with the specific spectrum of morphological changes occurring during infusion of the different types of media.

The morphological alterations occurring during infusion were most likely direct results of the increased intraluminal pressure.

Thus, dilation of ducts and acini increased successively during filling of the gland and the severity of the alterations correlated with the amount of pressure induced for the two different kinds of media. Luminal dilation has been seen previously following retrograde infusion under pressure into salivary glands (Parsons and Garrett, 1977). Retrograde infusion during increased intraluminal pressure also causes dilation of the proximal tubule of the kidney (Ottosen, 1976). Luminal dilation seen in salivary glands (Tamarin, 1967; 1971), the exocrine pancreas (Edlund et al., 1963; Metz et al., 1978) and the liver (Metz et al., 1977; Koga and Todo, 1978) following ductal ligation, are probably partly caused by the resulting increase in pressure.

The pressure induced during infusion is most likely dependent on the rate of infusion. A lower increase in pressure, and a lack of complete ductal filling as seen radiographically, were noted at lower infusion rates using the water-based medium (unpublished observations). These phenomena are probably due to a rapid removal of the compound from the luminal space. Using the lipid-based medium the developing pressure and the resulting morphological alterations would, most likely have been similar at comparable degrees of filling. However, it is possible that the alterations would have been somewhat milder if the induced pressure increase was less sudden.

The initial changes were apparently caused by back pressure of saliva present in the gland, since they were seen prior to the

presence of contrast medium. This was determined by correlation to the sialographic appearance and by lack of tracer in the tissue. Increased pressure from saliva alone, induced by hydrostatic forces, is known to cause similar changes (Emmelin et al., 1977).

Differences in pressure and resulting morphological alterations induced at later stages during infusion of the two contrast media can be explained by differences in their distribution in the tissue. This is a consequence of the differences in solubility and viscosity between Renografin and Lipiodol.

The water-based medium mixes with saliva present in the gland immediately after reaching the lumen (Goebel, 1977). Its subsequent leakage into the intercellular spaces, shown by the distribution of the tracer, prevents further build up of pressure in the lumina. The similarities of both the pressure development and the induced morphological alterations to those obtained during infusion of saline solution, strongly indicate the significance of the physical properties of the infused medium.

The high viscosity of the lipid-based medium and its insolubility in the tissue will likewise determine the distribution. The much higher increase in pressure and the induced severe alterations could be explained by its confinement to the luminal space. Thus, lack of leakage and diffusion of this medium will lead to a successive build up of luminal pressure resulting in the pronounced distension, comprising vacuole formation and compression of parenchymal cells, observed at later stages of filling. The limited distribution of saliva in the lumina, most likely reflects

displacement by infused Lipiodol (Goebel, 1977). The electron dense border seen in the dilated lumina could thus constitute secretory proteins aggregated at the saliva-medium interface; and the empty area could represent space previously occupied by the lipid-based material.

These differences in distribution of the two media can explain the differences in the radiographic appearance. A rapid penetration of the water-based medium throughout the tissue, via the junctional complexes, agrees with the diffuse opacification noted radiographically. An intraluminal distribution of the lipid-based medium correlates with the well defined outline of the gland seen in the sialogram.

This indicates that, although the same four stages of filling can be distinguished, they do not represent the same localization of contrast medium in the tissue. During ductal filling, both media are primarily located in the interlobular and major intralobular ducts, whereas at later stages, in addition, smaller ducts and acinar lumina contain contrast medium (Samuel, 1950; Ollerenshaw and Rose, 1951; Lowman and Cheng, 1976). Using the water-soluble medium, the change in appearance obtained during parenchymal filling is probably due to its presence in the intercellular spaces. The following stages represent the distribution of the infused medium at the basal surface of the various cells and in the connective tissue.

The sialographic appearance obtained during parenchymal filling using the lipid-based medium, is probably partly due to

pronounced dilation of the intralobular ducts and acini. The differences in the developing pressure at the last stage do not allow for a direct correlation between the sialographic appearance and the morphological alterations. The morphological similarities between Sprague-Dawley (Tamarin and Sreebny, 1965) and Wistar (Fukuda, 1967; Tuch and Matthiesen, 1980) rat submandibular glands, and the diversity of morphological alterations observed at all stages of filling, suggest that the same type of alterations are present in the two strains. Thus, the well defined punctation observed radiographically at later stages of filling probably constitutes accumulation of contrast medium in successively larger groups of dilated lumina and vacuoles developing in the parenchyma, or in areas of total parenchymal destruction. This suggests that alterations induced by high pressure during filling of the gland with Lipiodol can be severe enough to significantly alter the sialographic appearance. The appearance of sialectasis can be obtained by disruption of striated ducts induced after filling with this medium (Garrett et al., 1976).

Differences in the effects of the increased intraluminal pressure on various types of parenchymal components were apparent at all stages of filling. This could be due to actual differences in the pressure exerted in different segments, depending on length and width of the luminal space. The excretory duct and the larger interlobular ducts were generally unchanged whereas more severe alterations were seen in intralobular ducts and acini. Using the

water-soluble medium, the pressure developing in the various segments is most likely dependent on the permeability of the junctional complexes. Variations in permeability exist both between junctional complexes in different organs (Friend and Gilula, 1972; Claude and Goodenough, 1973) and between different cell types in the same organ (Hirokawa, 1980) as suggested by differences in their morphological appearance. The normal appearance of the acini throughout infusion of both water-based medium and saline solution, and the large amount of tracer in the intercellular spaces in this segment, are indicative of a high permeability of the junctional complexes. Relatively high penetration through junctional complexes of salivary gland acini has previously been noted in various types of tracer studies (Garrett and Parsons, 1976; Parsons and Garrett, 1977; Garrett et al., 1981, 1982). Although, following intravenous administration, horseradish peroxidase penetration between acinar cells in the rat submandibular gland is not prominent (Yoshida et al., 1974), the luminal pressure induced during infusion could increase the permeability (Lorentz et al., 1972; Bulger et al., 1974; Ottosen, 1976). The severe alterations induced in the acini during infusion of the lipid-based medium further stress the importance of permeability in influencing the resulting alterations.

Variations in the damage induced in the different experiments could further be due to differences in resistance to pressure between the various segments. The acini, showing most severe alteration following infusion of the lipid-based compound, are known to be more sensitive than the ducts to a variety of insults. Thus, they are more

sensitive to irradiation (Sholley et al., 1974; Nicolatou, 1981) and starvation (Tamarin and Sreebny, 1962) and react earlier to duct ligation (Tamarin, 1967). The specific responses of the various duct segments i.e. scalloping of the granular and striated duct lumina, with largely intact junctional complexes, and irregular dilation of the intercalated ducts with widening of desmosomes, could reflect specific characteristics of the different cell types. The persistence of these appearances of the luminal border through various stages of filling during infusion implies differences in reaction to pressure.

The recovery of the glandular tissue was also dependent on both the degree of filling and the medium used. Variations in the severity of the alterations in different areas noted throughout the recovery period, most likely correspond to the initial damage.

Most of the alterations noted during the recovery period are primarily directly or indirectly caused by the increased luminal pressure induced by infusion, and were consistently more frequent and more pronounced in glands infused with the lipid-soluble medium. The luminal dilation and the characteristic appearances of the luminal border of the various segments noted at early recovery times, were similar to those induced during infusion, although somewhat less prominent. Some decrease in distension most likely occur as soon as the pressure is removed from the luminal space (Emmelin et al., 1977). Persistence of the irregularity of the apical surface of the lining cells, including the apparent vacuoles in the apical cytoplasm, could reflect damages to the luminal membrane.

Further increase of luminal space and leakage through junctional complexes in damaged ducts and acini during secretion, could partly account for the lower flow rates observed at early times. Reduced salivary flow has previously been reported after release of ductal ligation (Martinez et al., 1982) and during increased intraluminal pressure (Siegel, 1976; Dawes et al., 1980). The extensive structural alterations in the ducts, seen after heavy parenchymal filling with Lipiodol, could explain the increase in K^+ levels in saliva from these glands at 20 h.

Alterations in the secretory mechanism could account for a number of the changes seen in the acinar cells. Confluence of secretory granules and vacuole formation were probably induced secondarily, as they were not observed immediately after infusion. Similar vacuoles are seen in salivary glands after starvation (Hand, 1972), in Streptozotocin-induced diabetes (Cutler, et al., 1979), after repeated administration of autonomic antagonists (Donath, et al., 1974) and following stimulation preceded by degranulation (Garrett, et al., 1978). These reactions may be caused by changes in the secretory mechanism such as disruption of the normal exocytotic process due to alterations in the apical membrane. The observed discharge of secretory granules at the lateral cell membrane could also result from such changes.

More severe alterations of the luminal membrane, caused by a higher intraglandular pressure, could explain the more pronounced changes observed in glands infused with the lipid-based medium.

Accumulation of secretory granules in acinar cells of these glands, previously seen following sympathectomy (Garrett and Harrop, 1976) could also be due to alterations in the secretory mechanism. Formation of autophagic vacuoles, frequently observed, strongly indicates severe cell damage, such as occurs after X-irradiation (Pratt and Sodicoff, 1972; Sholley et al., 1974; Sodicoff et al., 1974; Chomette et al., 1981; Nicolatou, 1981) and duct ligation (Tamarin, 1967; Churg and Richter, 1971; Donath et al., 1973) of exocrine glands. It could partly be a result of functional alterations, specifically in the secretory mechanism, since it occurs following administration of sympathetic antagonists (Donath, 1973) and in other conditions known to affect secretion (Han, 1967; Hand, 1972; Kelly et al., 1978; Hand and Ho, 1981).

The observed inflammatory reaction, primarily a direct result of the cellular damage and functional alterations, was similar to the reaction seen after duct ligation (Bhaskar et al., 1966; Harrison and Garrett, 1976) and could partly be due to retention of secretory product (Tamarin, 1979). The stronger inflammatory response and the localization of the inflammatory cells observed after infusion of the lipid-based medium is in agreement with more severe alterations. Accumulation of inflammatory cells within the basement membrane has been seen in number of studies involving experimentally induced cellular injury (Hand, 1973; Schneyer and Wilborn, 1976; Oliver et al., 1979; Hand and Ho, 1981) including X-irradiation (Pratt and Sodicoff, 1972; Sodicoff et al., 1974) and duct ligation (Harrison and

Garrett, 1976; Tamarin, 1979). The clusters of macrophages surrounding damaged cells, seen in these glands, probably represent sites for phagocytosis and breakdown of cellular debris.

The stronger reaction and the longer lasting changes seen in glands infused with the lipid-based medium are partly due to its retention in the tissue (Trester, 1968; Mason and Chisholm, 1975). The vacuoles present in parenchymal cells and macrophages could represent sites for breakdown of the contained electron dense material, probably constituting remnants of the contrast medium. The macrophage-enclosed spaces containing electron dense material could have a similar function. In addition, they may represent the same type of response as the larger cyst-like spaces primarily seen in the interlobular connective tissue, which most likely reflect a granulomatous reaction. Such alterations, previously observed following retrograde filling of salivary glands (Epsteen and Bendix, 1954; Epsteen, 1956), develop due to retention of lipid-based medium (Mandel and Baurmash, 1962; Lilly et al., 1968).

The toxicity of diatrizoate, the base in Renografin, is relatively low (Hoppe et al., 1956; Hoppe, 1959; Hoppe and Archer, 1960) but the compound can cause increased permeability of endothelium and perivascular edema (Harrington and Wiedeman, 1965). Such reactions, occurring during ductal administration, although not apparent at the time, could contribute to the changes observed during the recovery period.

The alterations induced by the presence of the water-based medium are most likely of minor significance compared to the reaction caused by the lipid-based compound. These differences are certainly of importance in regard to the long term effects induced by infusion of the two types of media. In addition, both the high pressure developing in the luminal system during infusion of the lipid based medium, and its retention in the tissue, may cause alterations or temporary obstruction of the blood flow (Lubarsky and Nicoll, 1968). The intracellular transport and exocytosis of secretory proteins are highly energy dependent (Jamieson and Palade, 1971). A resulting hypoxia may have an effect on the secretory process and induce changes in the parenchymal tissue (Morawa and Han, 1974), and could also contribute to the more severe alterations induced by using the lipid-based medium.

The apparently enhanced differences between the two degrees of filling in the long term studies could be due to additional alterations occurring during the recovery period (Bhaskar et al., 1966). This could partly reflect the amount of contrast medium infused (Morino et al., 1959) and the time of exposure (Olsson, 1954). In addition, the recovery could be dependent on the amount of tissue affected initially.

The atrophy noted after overfilling with either medium, comprising reduction in size of the parenchymal cells and an increase in the connective tissue stroma, has been seen in salivary glands with decreased function after liquid diet (Hall and Schneyer,

1964; Sreebny and Johnson, 1968; Johnson and Sreebny, 1971; Hand and Ho, 1981). Both atrophy (Sodicoff et al., 1974) and connective tissue proliferation (Nicolatou, 1981) observed following X-irradiation could be due to degenerative and functional alterations. The differences in severity of the changes between glands, and between different areas in the same gland, could account for the absence of alterations in flow or saliva composition at this time in glands included in the functional study. The few and mostly insignificant alterations noted in the functional study may be due to that some parts of each gland, remaining relatively intact, could provide for a normal secretion. In addition, it is possible that a compensatory mechanism is responsible for continuous function of the gland.

Necrosis was rarely seen, even in the most affected glands and mitosis was not increased compared to the controls (unpublished observation). This indicates that an actual recovery of the parenchymal cells occurs, rather than a regeneration. Redifferentiation of preexisting cell population has previously been noted in rat submandibular glands severely altered by duct ligation (Tamarin, 1971 a, b). The presence of numerous granular duct cells in the acinar-intercalated duct junction (Qwarnstrom and Hand, 1983) after heavy parenchymal filling with Lipiodol suggests that some regeneration occurs in the most severely altered glands.

The alterations observed in these studies were primarily a result of the increase in pressure induced during infusion. Infusion

of radiographic contrast medium into human salivary glands also result in an increase in pressure (Zijlstra and Ten Bosch, 1975; Ferguson et al., 1977). A direct extrapolation to the clinical situation can not be made from the results obtained in these studies. The types of alterations occurring in the human salivary gland during sialography will, however, presumably be similar to the ones described, since such responses have been shown in various exocrine glands from different species in similar experimental conditions cited in this study. Both the distribution of the infused media and the severity and sequence of the changes induced during clinical sialography may be quite different, due to histological dissimilarities between human (Mason and Chisholm, 1975) and rat submandibular glands (Tamarin and Sreebny, 1965). In addition, reflex stimulation of myoepithelial cells in the unanesthetized patient, could have an effect on the alterations, by reducing the distensibility in acini and intercalated ducts (Emmelin et al., 1977). Pathological alterations present in salivary glands prior to the sialographic examination will presumably also influence the effect of the procedure.

Using the experimental model it was possible to determine the type of alterations that can occur in a salivary gland due to retrograde infusion of contrast medium, and under what conditions the sialographic examination is less harmful as regards retrograde infusion and filling of the gland.

LEGENDS FOR FIGURES AND TABLES

Figure 1.

Pressure curves obtained during infusion (0.008 ml/min) of (a) Urografin 60%, (b) isolonic saline (0.9%) and (c) Lipiodol UF into the submandibular gland of Wistar rats (from the radiographic study, I). The four stages represent, (A,A¹) ductal filling, (B,B¹) parenchymal filling, (C,C¹) heavy parenchymal filling and (D,D¹) total opacification of the gland.

Figure 2.

Pressure development in the monitoring system during infusion of Renografin-60 (B,D) and Lipiodol UF (A,C) at a rate of 0.008 ml/min.

A,B: After closing the tubing at the level of the main excretory duct of the gland.

C,D: When the system is open to the air at the part normally inserted into the gland.

Table I

The most prominent alterations observed in the glandular tissue at various degrees of filling with Renografin-60 and Lipiodol UF, respectively. The four parts of the pressure curves correspond approximately to the four different degrees of filling of the gland described in the radiographic study (I).

Table II a & b

The most prominent alterations observed in the glandular tissue at various times after filling with Renografin-60 and Lipiodol UF. The two parts of the curve correspond approximately to ductal and slight parenchymal filling and heavy parenchymal filling, respectively.

Figure 1

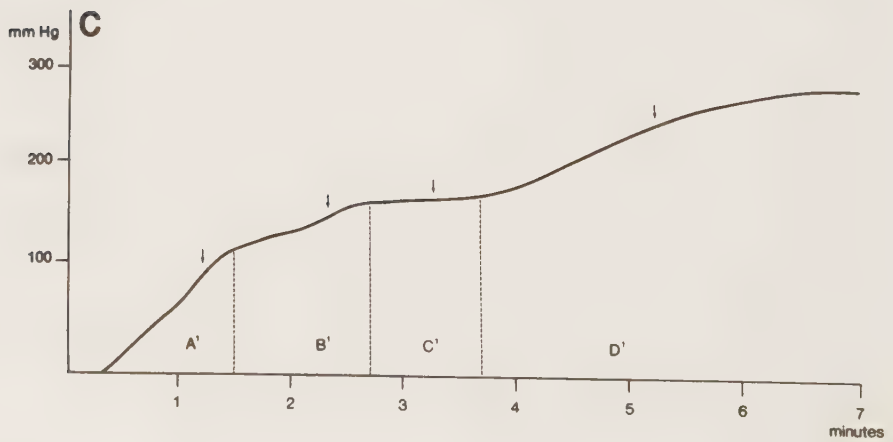
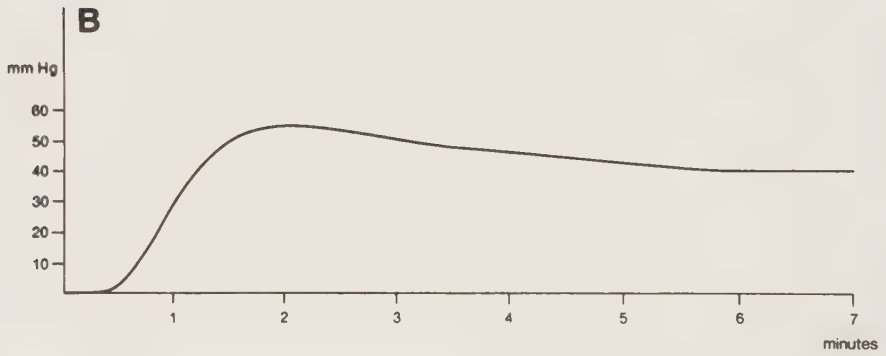
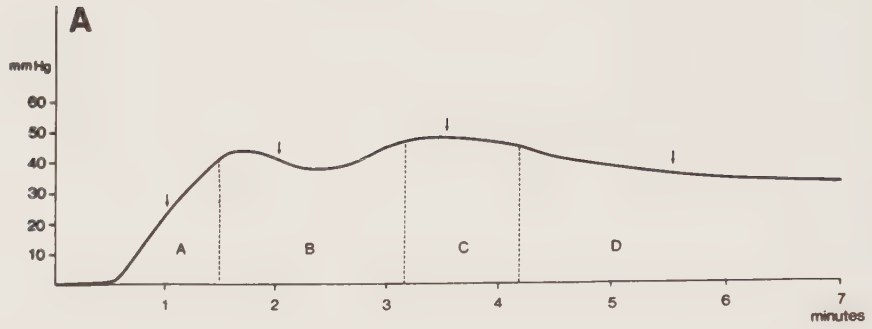


Figure 2

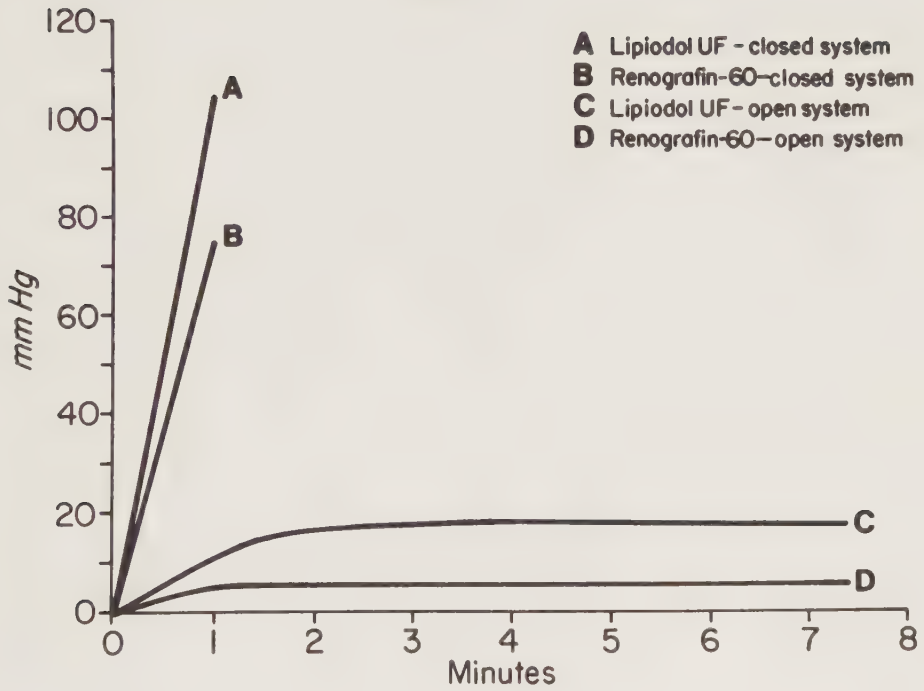


TABLE 1

Part of pressure curve	Renografin-60	Part of pressure curve	Lipiodol UF
Initial increase:	Successive dilation of intralobular ducts. Scalloping of the luminal border of the granular and striated ducts. Irregularity of luminal border of intercalated ducts. HRP appears in the tissue in the latter part.	Initial increase:	Successive dilation of intralobular ducts. Scalloping of the luminal border of granular and striated ducts. Irregular dilation of intercalated ducts and widening of the desmosomes.
Peak 1 top & valley:	Further duct dilation. Widening of the desmosomes between intercalated duct cells. HRP in the intercellular spaces and some at the basal surface of the parenchymal cells.	Plateau 1 & 2nd increase:	Further duct dilation. Widening of the desmosomes more pronounced. Dilation of acinar lumina with widening of the intercellular canaliculi.
Peak 2 top:	Duct dilation more prominent and more common. Widening of desmosomes between intercalated duct cells more common. HRP accumulating basally, in the connective tissue, and some in the walls of blood and lymphatic vessels.	Main peak: (Corresponding to the second plateau using the Wistar rat)	Luminal dilation in all segments pronounced. Widening of the desmosomes between intercalated duct cells more pronounced and more common. Vacuoles in continuity with the luminal space developing, primarily in the intercalated duct cells. Compression of the parenchymal cells, in particular intercalated duct and acinar cells.
Final plateau:	Duct dilation prominent and common. Widening of desmosomes between intercalated duct cells more common. HRP throughout connective tissue stroma and present in blood and lymphatic vessels.	Final plateau: (Corresponding to final increase using the Wistar rat)	Secretory product present in part of the dilated lumina, lined by an electron dense interface. Further dilation of lumina. Increase in number and size of cytoplasmic vacuoles, partially filled with secretory product. Pronounced compression and deformation of, in particular intercalated duct and acinar cells. Widening of the interlobular connective tissue spaces.

TABLE IIa
Renografin-60

Part of Pressure Curve	<u>Beginning of first peak</u>	<u>Beginning of second peak</u>
Time after Infusion		
10 h	Luminal dilation of intra- lobular ducts with scalloping and irregularity of luminal border.	Luminal dilation of intralobular ducts with scalloping and irregularity of luminal border. Confluence of secretory granules in acinar cells. Inflammatory cell infiltrate.
20 h	Scalloping and irregularity of luminal border of intra- lobular ducts. Luminal dilation of acini, occasionally. Inflammatory cell infiltrate peak.	Luminal dilation of intralobular ducts with scalloping/irregularity of luminal border. Luminal dilation of acini. Confluence of secretory granules in acinar cells. Lateral release of secretory granules, occasionally. Inflammatory cell infiltrate, peak.
30 h	Some scalloping/irregularity of luminal border. Reduced inflammatory infiltrate. Generally, normal appearance of tissue.	Luminal dilation of intralobular ducts with scalloping/irregularity of the luminal border. Confluence of secretory granules and vacuole formation in acinar cells. Inflammatory cell infiltrate.
4 d		Some reduction in size of parenchymal cells. Reduced number of secretory granules in acinar and granular duct cells. Inflammatory cells.
7 d		Atrophy of parenchymal cells in some areas. Reduced number of secretory granules in acinar and granular duct cells. Proportional increase in connective tissue stroma. Increase in the number of small blood vessels. Inflammatory cells.
10 d		Generally normal appearance of tissue. Some atrophy.

TABLE II b
Lipiodol UF

Part of pressure curve	Beginning of first plateau	Top of major peak (Corresponding to the second plateau using the Wistar rat.)
Time after Infusion		
10 h	Luminal dilation of intra- lobular ducts with scalloping and irregularity of luminal border. Confluence of secretory granules in acinar cells. Widening of intercellular spaces between parenchymal cells. Inflammatory cell infiltrate.	Luminal dilation of intralobular ducts with scalloping and irregularity of the luminal border. Widening of acinar lumina. Confluence of secretory granules in acinar cells. Widening of intercellular spaces between parenchymal cells. Inflammatory cell infiltrate.
20 h	Luminal dilation of intra- lobular ducts with scalloping/ irregularity of the luminal border. Basal vacuoles in duct cells. Widening of acinar lumina. Confluence of secretory granules in acini. Autophagic vacuole formation. Widening of intercellular spaces. Inflammatory cell infiltrate, peak.	Luminal dilation of intralobular ducts with scalloping/irregularity of the luminal border. Basal vacuoles in duct cells. Widening of acinar lumina. Confluence of secretory granules and vacuole formation in acinar cells. Autophagic vacuole formation. Widening of intercellular spaces. Inflammatory cell infiltrate, peak. Cyst-like spaces surrounded by inflammatory cells in interlobular connective tissue.
30 h	Less pronounced morpho- logical alterations. Inflammatory cell infiltrate, reduced.	Vacuoles in acinar cells. Autophagic vacuoles in parenchymal cells. Widening of intercellular spaces. Inflammatory cells intercellularly in parenchyma. Inflammatory-cell-enclosed spaces in parenchyma containing electron dense material.
4 d	Generally normal appearance of tissue.	Vacuoles in parenchymal cells, some containing electron dense material. Autophagic vacuoles in parenchymal cells. Reduced size and compression of duct and acinar cells. Inflammatory cells intercellularly. Inflammatory-cell-enclosed spaces in parenchyma containing electron dense material.
7 d		Atrophy and deformation of some parenchymal cells. Increase in connective tissue stroma. Inflammatory-cell-enclosed spaces in parenchyma. Inflammatory cells surrounding damaged parenchymal cells.
10 d		Generally normal appearance of tissue. Some areas parenchymal atrophy. Reduced inflammatory cell infiltrate.

SUMMARY AND CONCLUSIONS

The morphological and functional effects of retrograde infusion on salivary glands were examined, using the rat submandibular gland as an experimental model. A system for sialography was designed, involving retrograde infusion during continuous pressure monitoring. Morphological alterations induced by retrograde infusion of water- and lipid-soluble media were examined by light and electron microscopy after various degrees of filling of the gland. The immediate alterations as well as the recovery and long term effects were evaluated. Similarly the effects of infusion of isotonic saline were studied. In addition, the distribution and fate of the infused media were determined. The function of the glands at different times following infusion of the contrast media was also evaluated.

1. Various degrees of filling of the gland seen in the sialogram could be correlated with specific parts of characteristic pressure curves obtained during continuous infusion of water- and lipid-soluble radiographic contrast medium.
2. Retrograde infusion of both contrast media and the isotonic saline solution led to an uneven distribution throughout the tissue. Variations in the severity of the induced alterations in different parts of the gland were noted at all stages of filling.

3. The immediate alterations induced by infusion of either media primarily comprised dilation of the luminal system and were directly caused by the increased intraluminal pressure induced during infusion.
4. The severity of the alterations was dependent on the degree of filling of the gland. As infusion continued the induced changes successively became more pronounced and a larger proportion of the tissue was affected after longer infusion.
5. The distribution in the tissue differed between the two contrast media. The water-soluble medium, as the isotonic saline solution, leaked out into the intercellular spaces and the surrounding connective tissue, as demonstrated by localization of a soluble phase tracer, horseradish peroxidase, dissolved in the medium. The lipid-soluble medium remained in the luminal space.
6. The alterations induced by infusion of the lipid-soluble medium were consistently more severe than those seen using the water-soluble medium. In addition to dilation of the luminal space, infusion of the lipid-based substance resulted in the formation of large vacuoles in continuity with the lumen, and compression of the parenchyma. This is thought to be directly related to the higher pressure induced.

7. The recovery and the long term effects were, as the immediate changes, dependent on both the degree of filling and the medium used. Variations in the severity of the alterations seen in different areas probably corresponded to differences in the initial alterations.
8. The alterations present during the recovery period were primarily directly or indirectly, caused by the increased intraluminal pressure. The presence of the infused contrast medium was also of importance for the observed alterations.
9. Ductal filling with either medium resulted in a relatively fast recovery with a successive decrease in luminal diameter. After prolonged infusion, the recovery was much slower and numerous additional alterations occurred. Alterations in the acini comprised confluence of secretory granules and vacuole formation. In some of the glands subjected to heavy parenchymal filling, atrophy of the parenchyma and a proliferation of connective tissue were observed at later times.
10. The more severe alterations induced using the lipid-based medium are due to the higher pressure administered during infusion. In addition, retention of the medium was of significance, and caused granulomatous changes during the recovery period.

11. An inflammatory infiltrate was observed in all glands during the recovery period. It was more prominent in the glands subjected to heavy parenchymal filling, in particular after infusion of Lipiodol.

12. Functional alterations as reflected in salivary flow rate and saliva composition, were few. No significant alterations occurred after ductal filling. The most noticable change was a decrease in flow rate, observed after heavy parenchymal filling with either medium.

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Sialography of the submandibular gland in the rat

An experimental model including measurement of intraglandular pressure

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KEY WORDS: *Rats, radiography, sialography, submandibular gland*

Sialography was carried out on submandibular glands of rats. During infusion of water-soluble and fat-soluble contrast media, the intraglandular pressure was recorded. The pressure development during infusion of isotonic saline solution was also studied. Reproducible pressure curves characterizing each medium were obtained. It was possible to relate certain sialographic stages to certain phases of the pressure curves. The experimental model should offer new possibilities in sialographic research.

Radiographic contrast examination of salivary glands, sialography, has gained increasing clinical acceptance as a diagnostic tool in salivary gland disease (12). This has stimulated the development of new and enhanced sialographic techniques. In particular, efforts have been made to control the infusion of contrast medium into the glands. Thus, hydrostatic (gravity-fed) sialography (1, 2, 9, 14, 15) and isobaric sialography (19) have evolved. Earlier, Jaensch (11) in clinical examinations had utilized equipment which allowed measuring of infusion pressure and rate of infusion of the contrast medium.

Zijlstra & Ten Bosch (24) developed a technique which enabled a continuous observation and recording of the injection pressure and a determination of equilibrium pressure, i.e. a pressure existing in the salivary gland just before ligation of the duct prior to radiography.

Recently, Ferguson et al. (6) described a clinical method for sialography in which a constant flow rate was adopted and the ductal pressure monitored throughout.

In order to facilitate a systematic study of how factors such as infusion pressure, rate of infusion of contrast medium and rate of resorption of the medium affect the degree of gland-

ular filling, we have developed a procedure for experimental sialography on the rat submandibular gland.

The present paper describes the experimental model and reports that it is possible to relate characteristic radiographic appearances of the gland to the development of the intraglandular pressure during infusion of contrast medium.

MATERIAL AND METHODS

Preparation technique. Twenty-four glands from 16 Wistar rats, weighing 250–500 g, were used. General anesthesia was obtained by intraperitoneal injection of chloral hydrate in an initial dose of 240 mg/kg body weight. Additional doses were given when required. The animals were tracheotomized and a polyethylene catheter was inserted in the femoral vein. The submandibular excretory ducts were dissected extraorally and cannulated as close as possible to their orifices in the mouth using fine glass cannulae, outer diameter 0.5 mm, according to the technique of Ohlin (13). To ascertain that the cannulae were properly positioned and that there were no obstructions in salivary flow, the secretory response to the parasympathomimet-

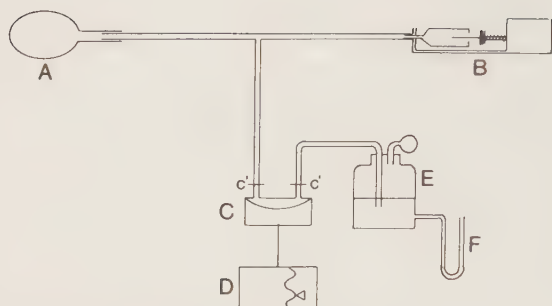


Fig. 1. Infusion and pressure recording device. A. Submandibular gland; B. Syringe with contrast medium fitted in infusion pump; C. Displacement transducer supplied with two-way taps (c'); D. Recorder; E. Pressure bottle; F. Mercury manometer.

ic drug methacholine (2–5 $\mu\text{g/kg}$ body weight i.v.) was checked. Salivary secretion was also evoked approximately 10 min before start of infusion of contrast material.

Infusion and pressure recording device. The glass cannula in the salivary duct (Fig. 1) was connected to one end of a T-shaped system of polyethylene tubing, the other two ends of which were connected to an infusion pump and a displacement transducer. The displacement transducer was fitted with two-way taps and connected to a pen-recorder. A syringe containing the contrast medium was fitted to the pump; the part of the tubing between pump and gland was filled with the contrast medium and the other parts of the system with saline solution (0.9%). The displacement transducer was also connected to a pressure bottle supplied with a pumping balloon and a mercury manometer. Closing the inlet from the gland, the recording system could be calibrated by using the pressure bottle system. During the experiments, the inlet from the gland was opened and the connection to the calibrating system was closed. The pressure measuring device was operated in accordance with the principles adopted by Emelin et al (3).

Sialography. The animals were positioned supine on an acrylic board containing a film holder. The board with the rat was placed on the table of a Lysholm precision apparatus for skull

and skeletal radiography and the glass cannula was connected with the tubing from the infusion pump. To project the gland as free as possible from the base of the skull, the x-ray beam, diameter 40 mm, was angled 17° medially and 10° anteriorly. Focus/film distance was 750 mm and the x-rays, 55 kV and 120 mAs, were emitted from a Siemens tube with rotating anode, focal spot $0.6 \times 0.6 \text{ mm}^2$. A non-screen film, Agfa Gevaert D4p, was used.

One of two contrast media were infused: Urografin 60% (Schering), which is water-soluble and the fat-soluble Lipiodol UF (Guerbet). The iodine content of Urografin 60% and Lipiodol UF is 292 and 480 mg/ml, respectively. The viscosity amounts to approximately 4 mPa·s for Urografin 60% and 25 mPa·s for Lipiodol UF at 36°C . In addition, some glands were infused with isotonic saline solution only.

Infusion rate and duration were based on preliminary experiments. The rate was approximately 0.008 ml/min. The duration was determined by the sialographic appearance. In no case was the infusion disconnected until the gland was overfilled with contrast material. Infusion time was measured with a stopwatch beginning when the infusion pump was activated. In each experiment it amounted to approximately 7 min.

Flow of Urografin 60% and Lipiodol UF through the open system including glass cannula caused an increase in pressure amounting to 6 and 18 mmHg respectively, whereas passage of saline solution did not result in a pressure increase.

One radiograph was always obtained before start of infusion. During continuous infusion the intraglandular pressure was monitored and radiographs were taken at 30 s intervals from zero time. The moments of exposure were marked on the time axis of the pressure curves.

RESULTS

Urografin 60%

Pressure versus time curves. Eight glands from six animals were infused with Urografin 60%. At the beginning of infusion, there was an interval of approximately twenty seconds before the

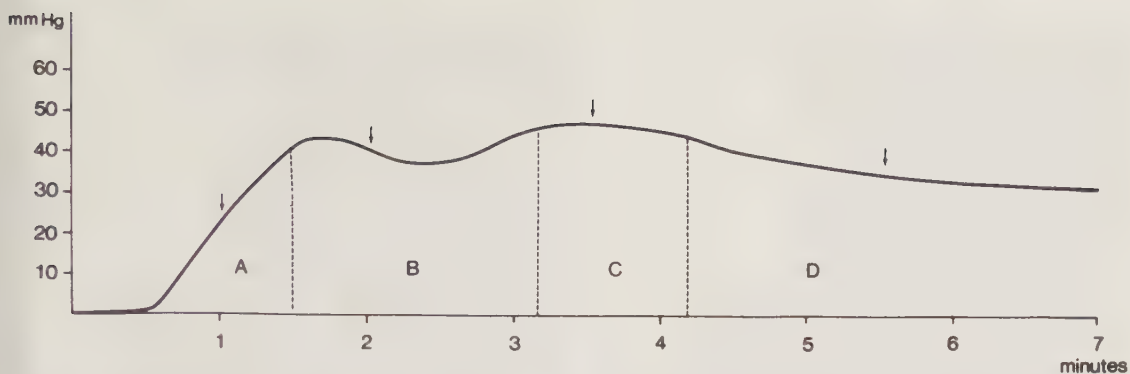


Fig. 2. Recording of intraglandular pressure from an experiment using Urografin 60%. A-D represent pressure phases with characteristic sialographic ap-

pearances illustrated in Fig. 3. Arrows indicate when radiographs shown in Fig. 3 were taken.

pressure started to rise. The increase in pressure was fairly rapid until a first peak was reached. The following pressure fall was only slight. Eventually, the pressure built up to a second, generally higher peak after which a gradual decrease occurred until the pressure remained constant despite continued infusion. The top of the first and second peak was reached approximately 1 and 3 min, after start of infusion, respectively.

One of the curves is shown in Fig. 2. The pressure recordings from all the glands showed a uniform pattern, dissimilarities being minor. The mean pressure of the first and second peak was 40.6 ± 7.8 mmHg (means $\pm$ SD; range 30–52) and 45.2 ± 8.0 mmHg (range 34–58), respectively. In the valley between the peaks, the pressure dropped to a mean of 31.4 ± 8.9 mmHg (range 21–50). The mean pressure of the final plateau was 26.9 ± 9.2 mmHg (range 17–44).

Sialograms. The study of all the radiographs exposed during infusion revealed that for each gland there were four consecutive stages of contrast material distribution. In the first stage (Fig. 3a), the contrast medium seemed to be confined to the intraglandular ducts, in the second (Fig. 3b) so-called parenchymal filling appeared and in the third (Fig. 3c), single larger dots of contrast material were present. In the fourth stage (Fig. 3d) there was a confluence of the dots.

Comparison sialograms/pressure curves. Since the moments of exposure were marked on the time axis of each pressure curve, the appearance of the sialograms could be related to the pressure existing at the time of exposure. It was found that the four stages of glandular filling described above occurred during the four phases (A–D) of the pressure curve shown in Fig. 2.

Lipiodol UF

Pressure versus time curves. Eight glands from five animals were infused with Lipiodol UF. The resulting eight curves were not as consistent as those obtained after infusion of Urografin. It was not difficult, however, to identify some features common to all the Lipiodol curves. Thus, after start of infusion there was about a twenty second interval before the pressure started to rise. The pressure then increased rapidly to a level where the increase became very slow. Here, the pressure was sometimes even constant. This was followed by a marked rise in pressure to a second level, where the course of pressure development was similar to that of the first level. In most curves, the second level was more distinct than the first and also lasted longer. The following pressure rise was at first fairly rapid but became successively less pronounced, so that in some cases eventually no pressure change occurred. The midpoints of the plateaus were reached after 1.5–2 min and



Fig. 3. Sialograms obtained during infusion of Urografin 60%. a) Contrast medium confined to intraglandular ducts (phase A); b) So-called paren-

chymal filling (phase B); c) Larger, single dots of contrast material (phase C); d) Confluence of radiopaque dots with opacification of gland (phase D).

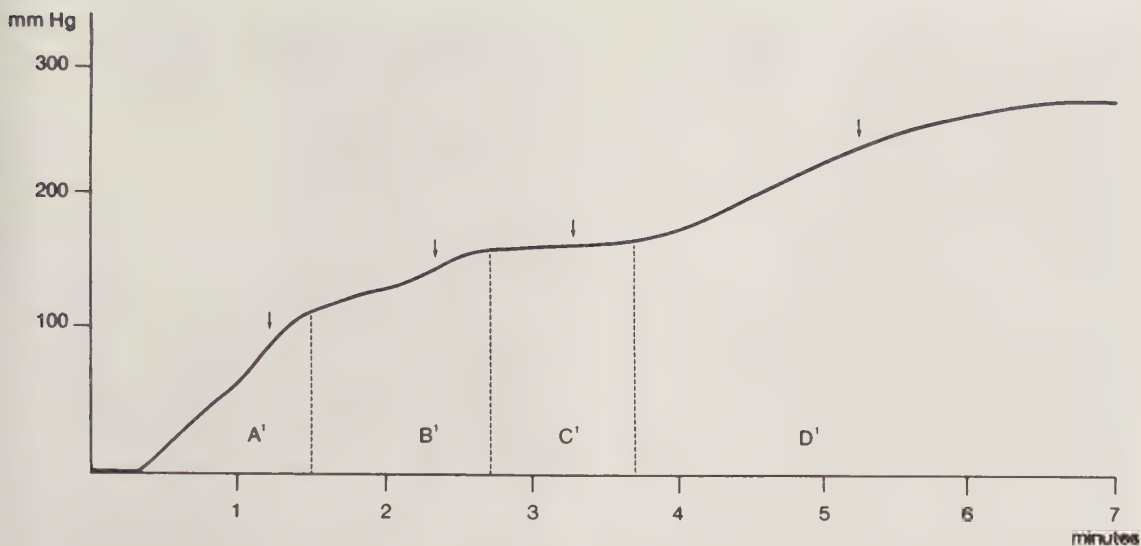


Fig. 4. Recording of intraglandular pressure from an experiment using Lipiodol UF. A¹-D¹ represent pressure phases with characteristic sialographic appearances illustrated in Fig. 5. Arrows indicate when radiographs shown in Fig. 5 were taken.

3–5.5 min, respectively. One of the Lipiodol curves is shown in Fig. 4.

The mean pressure of the first and second plateau was 142.5 ± 33.6 mmHg (range 103–190) and 194.8 ± 33.6 mmHg (range 161–239), respectively.

Sialograms. Infusion of Lipiodol resulted in a better contrast than the infusion of Urografin. Whereas the contours of the gland were poorly exhibited with Urografin, Lipiodol distinctly revealed its contours. It was possible to classify the stages of filling appearing during infusion of Lipiodol (Fig. 5) in the same way as described for Urografin.

Comparison sialograms/pressure curves. The times when the different sialographic appearances occurred corresponded uniformly to different phases of the pressure curve. In phase A¹ (Fig. 4), a gradual filling of the ducts became visible (Fig. 5a). During phase B¹, an increasing amount of contrast medium appeared between the ducts, “parenchymal filling”, (Fig. 5b). In the following phase, C¹, larger, single, well defined dots of contrast material were observed (Fig. 5c). Eventually, in phase D¹, the gland acquired the appearance shown in Fig. 5d.

Isotonic saline solution

Pressure versus time curves. Eight glands from 5 animals were infused with isotonic saline solution. The pressure development during infusion was essentially the same for the eight glands. A fairly rapid increase in pressure began about half a minute after start of infusion. A maximum was reached in 1–2.5 min, after which the pressure slowly decreased and eventually stabilized at a plateau. The mean of the maximum pressure was 42.1 ± 8.1 mmHg (range 29–56) and of the final plateau 32.4 ± 7.9 mmHg (range 21–44). One of the pressure curves is shown in Fig. 6.

DISCUSSION

The rat submandibular gland was chosen for experimental sialography since its morphology is well known (20, 23). Also the nervous control of its myoepithelial and secretory activity and blood flow has been documented (21, 22). The radiographic anatomy of the gland was recently studied *in vitro* by Qwarnström & Omnell (17). The present investigation shows that sialograms *in vivo* also exhibit sufficient quality to permit detailed visual interpretation.



Fig. 5. Sialograms obtained during infusion of Lipiodol UF. a) Contrast medium confined to intraglandular ducts (phase A¹); b) So-called parenchymal filling (phase B¹); c) Larger, single, well defined dots

(arrows) of contrast material (phase C¹); d) Overfilling of gland without total confluence of contrast medium (phase D¹).

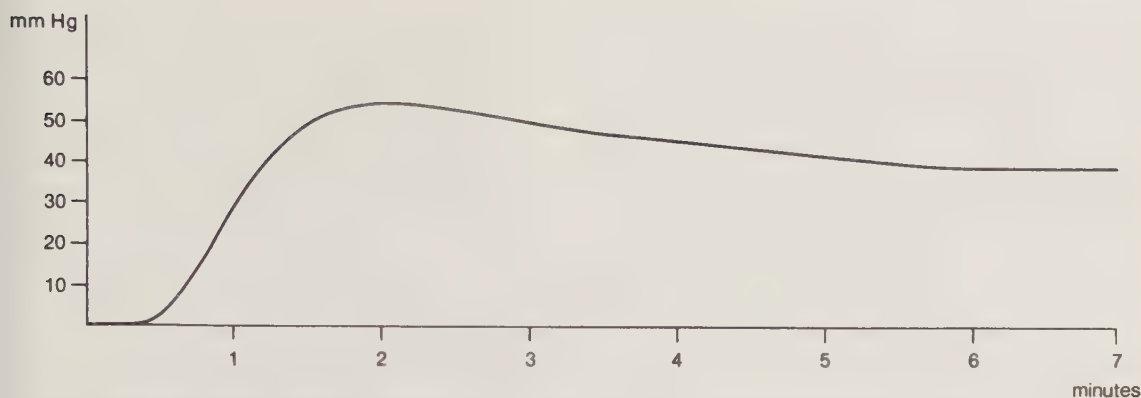


Fig. 6. Recording of intraglandular pressure from an experiment using isotonic saline solution.

Lipiodol UF, which has a higher iodine content than Urografin 60%, regularly produced better contrast. Differences in solubility and viscosity probably also contribute to the observed differences in contrast. A common feature for the two contrast media was four stages of glandular filling (Figs 3 and 5). In the first stage, the contrast media seemed to be retained within the lumina of the gland, whereas in the following stages the sialograms indicated also an extraluminal distribution. Recent histological studies, infusing water soluble contrast medium containing the tracer horseradish peroxidase, have shown that the medium rapidly leaks out from the lumina into the interstitial spaces (18). Similar results were obtained by Garrett & Parsons (8) and Parsons & Garrett (16) who demonstrated passage of luminally administered horseradish peroxidase through the intercellular junctions of adjacent acinar cells of dog and rabbit submandibular glands.

The radiographic appearances obtained in the third and fourth sialographic stages strongly suggest intraglandular damage. The correlation between the sialographic appearances and the phases of the pressure curves indicates that the intraglandular pressure development during infusion reflects morphological changes. It is known that ruptures of acini in cat sublingual gland occur due to an increase in pressure after duct ligation (10). Disruption of striated ducts in dog submandibular gland caused by secretion against a head of pressure has also been reported (4). Such changes are known to influence the sialographic picture of the gland.

Thus, experimentally induced ballooning disruption of striated ducts in dog submandibular gland may lead to the radiographic appearance of sialectasis (7).

Recently, electron microscopical studies (18) were carried out in order to determine the structural basis for the pressure changes occurring during infusion of water soluble contrast medium. Morphological changes consisting of luminal dilation were seen in all segments of the intraglobular duct system. However, the development of the intraglandular pressure could not be directly correlated with specific morphological alterations, but is thought to reflect all events occurring in the tissue at a particular time.

When comparing the pressure curves for Urografin, Lipiodol and saline solution, considerable differences were noticed. Common to all curves, however, was an initial short period before the pressure started to rise. This may reflect that the ductal volume of the resting gland, to some extent, can be increased without resulting in a rise in luminal pressure (5). The differences in pressure development are probably due to different timing of and different mechanisms for intraglandular distribution which likely are influenced by chemical and physical characteristics of the infused media. The recorded maximum pressure was essentially the same for the water soluble Urografin and for saline solution, whereas infusion of Lipiodol resulted in a much higher intraglandular pressure. Carrying out pressure monitored sialography in humans, Ferguson et al. (6) recorded a maximum

pressure which was approximately the same for water-soluble and fat-soluble contrast medium. The difference in species makes a comparison difficult, since the morphological characteristics of the gland as well as the relationship between its size and the infusion rate, also probably affect the appearance of the pressure curve.

This experimental model should offer new possibilities to study the interaction between parameters such as rate and duration of infusion, physical and chemical characteristics of the contrast media and intraglandular resorption, and how they affect the glandular filling and thus sialographic information. With slight alterations the procedure should also be applicable to clinical sialography in order to obtain a strictly controlled infusion.

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A LIGHT AND ELECTRON MICROSCOPIC STUDY OF THE DISTRIBUTION AND EFFECTS OF WATER-SOLUBLE RADIOGRAPHIC CONTRAST MEDIUM AFTER RETROGRADE INFUSION INTO THE RAT SUBMANDIBULAR GLAND

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Summary—For microscopic visualization, horseradish peroxidase (HRP; 10 mg/ml) was added to the contrast medium. Some glands were infused with saline solution containing HRP. During continuous infusion, the intraglandular pressure was recorded. Infusion was stopped at different phases of the pressure curve and the glands fixed by vascular perfusion. Morphological alterations, principally dilatation of the intralobular ducts, were most pronounced in the intercalated duct segment. HRP was first observed in the lumina of the intralobular ducts and acini but gained access to the intercellular spaces through the apical junctional complexes and subsequently surrounded various cellular components. Eventually the tracer was found in blood and lymphatic vessels. Because of an uneven distribution of the tracer and variation in the degree of filling in different areas of the same gland, the development of the intraglandular pressure could not be directly correlated with specific morphological alterations but is thought to reflect all events occurring in the tissue at a particular time.

INTRODUCTION

Retrograde injection of various substances into the salivary glands is done for experimental and diagnostic purposes. Experimentally, infusion through the main excretory duct has been used to study the local effect of pharmacologic agents upon different components of the salivary glands (Emmelin, Muren and Strömblad, 1954; Hedemark-Poulsen, 1974) and for inhibition of various glandular elements in ion-transport studies (Henriques, 1961, 1962; Miyoshi, 1963). Uptake of kallikrein in glandular tissue has been studied after this type of administration (Ørstavik, Gautvik and Nustad, 1980). Retrograde infusion of solutions has also been used to induce hydropic vacuolization and degenerative changes in the rat parotid gland (Sela *et al.*, 1977). The formation of endocytic vesicles and membrane recovery in exocrine acinar cells were studied using a lumenally administered tracer by Herzog and Farquhar (1977) and Oliver and Hand (1978).

Sialography involves filling, through the main excretory duct, the intraglandular ducts or the duct system and parenchyma with X-ray contrast medium. Little is known how factors such as infusion pressure, rate of infusion of contrast medium and rate of resorption of the medium affect the degree of filling of the duct system and the acini. An experimental model for sialography was developed (Qwarnström, Gjöstrup and Omnell, 1981) whereby different stages of glandular filling, as seen in the sialogram, could be correlated to the pressure developing in the gland during continuous infusion of contrast medium. The present investigation was carried out to determine the morphological alterations caused by the infusion and the distribution and fate of the infused media.

MATERIALS AND METHODS

A total of 14 male and female Sprague-Dawley rats weighing between 250 and 500 g were used. General anesthesia was obtained by intraperitoneal injection of chloral hydrate (400 mg/kg body wt). After tracheotomy, the left submandibular gland excretory duct was dissected extra-orally and cannulated approx. 5 mm anterior to the hilus using a fine glass cannula (external dia. = 0.5 mm) according to Ohlin (1965).

The intraglandular pressure was recorded during the experiments using a pressure monitoring system (Text Fig. 1) similar to that employed previously (Qwarnström *et al.*, 1981) and operated according to Emmelin, Garrett and Ohlin (1968). The glass cannula was connected to an infusion pump (Sage Instruments, syringe pump model 3413A) and to a displacement transducer (Statham 21207 P23 BB) which was connected to a recorder (Hewlett-Packard, 321) (Fig. 1). The syringe fitted to the pump and the polyethylene tubing connecting it with the gland were filled with the medium to be infused. Other parts of the system were filled with isotonic saline solution (0.9 per cent). The system was calibrated with a pressure bottle and a Hg manometer connected to the transducer.

One of the two media were infused through the main excretory duct: water-soluble contrast medium, Renografin-60 (Squibb; iodine content 288 mg/ml, viscosity 4 mPa · s at 36°C); or isotonic saline solution (0.9 per cent). For microscopic visualization of the media, an electron-dense tracer, horseradish peroxidase (HRP type II, Sigma Chemical Co.) was added (10 mg/ml). The infusion rate (0.008 ml/min) was the same as that used in the previous study and chosen because it gave an optimal filling of the duct system.

Before the infusion, the thoracic cavity of the anesthetized rat was opened, and the rat was artificially

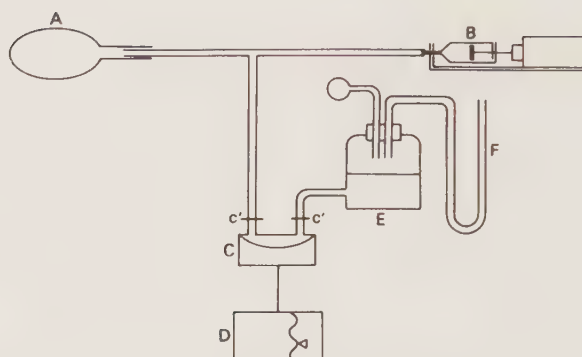


Fig. 1. Infusion and pressure monitoring device. A, submandibular gland; B, syringe, containing medium to be infused, mounted in infusion pump; C, displacement transducer with three way taps (c'); D, recorder; E, glass bottle with pumping bulb; F, mercury manometer.

respired with 95 per cent O_2 and 5 per cent CO_2 during the experiment. The infusion was stopped at different points on the pressure curves (Text Fig. 2a, b) and the glands were immediately fixed by vascular perfusion through the ascending aorta with a modified Karnovsky (1965) fixative containing 2 per cent glutaraldehyde and 2 per cent formaldehyde (Ladd Research Industries) in 0.1 M cacodylate buffer, pH 7.4, with 0.05 per cent calcium chloride. The two submandibular glands, the experimental and the contralateral used as a control, were removed and immersed in fresh fixative for approx. 3 h.

For routine morphologic studies, the perfused tissue was cut in 1 mm^3 pieces, rinsed in sucrose buffer (0.1 M cacodylate and 7 per cent sucrose) and stored overnight at 4°C . After postfixation at room temperature in 1 per cent osmium tetroxide in sucrose buffer (2 h), the tissue was rinsed and stained *en bloc* for 2 h with 0.5 per cent uranyl acetate in distilled water containing 5 per cent sucrose, dehydrated through a graded series of ethanols and embedded in Spurr (1969) resin.

Tissue for horseradish peroxidase (HRP) cytochemistry was cut into strips $1 \times 5\text{ mm}$, rinsed in su-

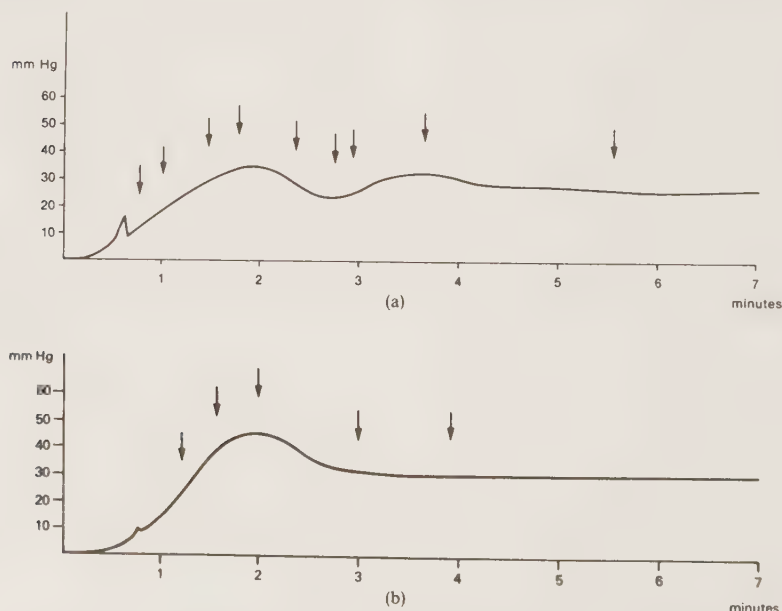


Fig. 2. (a) Typical intraglandular pressure development during infusion of water-soluble contrast medium, Renografin 60, containing HRP (10 mg/ml). Arrows indicate where infusion was stopped in the different experiments. (b) Typical intraglandular pressure development during infusion of isotonic saline solution containing HRP (10 mg/ml).

crose buffer and stored overnight at 4°C. The strips were chopped into 75 µm sections on a Smith-Farquhar TC2 tissue sectioner and incubated in a medium containing diaminobenzidine (1 mg/ml), 0.02 per cent hydrogen peroxide, 0.1 M cacodylate, pH 7.4, for 1 h at room temperature (Fahimi, 1970). After several rinses in sucrose buffer, the glands were postfixed in 1 per cent osmium tetroxide for 1 h and processed for electron microscopy. Additionally, a thin transverse slice from the centre of each gland was incubated for 2 h and embedded in JB-4 plastic (Polysciences) for light microscopic evaluation of HRP distribution.

Thick (1 µm) sections of embedded tissue, unstained or stained with toluidine blue, were examined by light microscopy. Thin sections of selected areas were cut with a diamond knife and mounted on bare copper grids. The sections were stained with uranyl acetate and lead citrate and examined in a Zeiss EM-10A electron microscope.

RESULTS

Renografin

The infusion of water-soluble contrast medium containing HRP resulted in an increase in the intraglandular pressure, beginning about 15 s after the start of the infusion (Fig. 2a). A sharp small peak, of at most 10 mmHg, occurred during a gradual increase to a first major peak of about 35 mmHg. A decrease of approx. 10 mmHg followed, after which the pressure increased to a second major peak also of about 35 mmHg. The pressure then decreased and finally leveled off at a plateau of approx. 30 mmHg where it remained despite continual infusion.

Infusion of the water-soluble contrast medium caused morphological changes consisting primarily of luminal dilatation of the intralobular ducts (compare Plate Figs 3 and 4). These changes initially appeared during the early part of the first pressure increase and were seen prior to detection of HRP reaction product in the tissue. All three components of the intralobular duct system (i.e. intercalated, granular and striated ducts) were affected, each presenting a characteristic appearance. The extent of damage in the different segments varied; however, in all three parts of the duct system, the changes successively became more frequent and more pronounced as infusion continued.

The most severe changes were in the intercalated ducts, particularly in the proximal part of the segment, adjacent to the acinar cells. The lumina of these ducts became irregularly enlarged (Plate Fig. 6). A considerable decrease in the height of the duct cells occurred, resulting in compression of the cytoplasmic constituents and flattened nuclei. A further increase in the luminal diameter, seen at later times appeared to cause a widening of the desmosomes between these duct cells (Fig. 6, inset). In some cases, this resulted in a loss of the central dense lamina. The luminal border of the granular ducts presented a scalloped appearance with the apical junctional regions projecting into the lumen (Plate Figs 4 and 7). The luminal membrane was pressed basally resulting in a compression of the apical granules. The striated ducts were least affected by the infusion. The lumen increased evenly in size with a minimal amount of scalloping and

marked flattening of the duct cells rarely occurred (Fig. 4).

Horseradish peroxidase first appeared in the tissue at the latter part of the first pressure increase. Light microscopic studies revealed an uneven distribution of reaction product throughout the gland (Plate Fig. 5). Heavily stained areas were present next to areas where no reaction product could be detected. Stained areas in the same gland also showed considerable differences in the amount of tracer present. At later times, a greater proportion of the tissue contained HRP, but the distribution remained uneven and areas with all degrees of filling were present.

Reaction product was first detected in the lumina of the different intralobular ducts, and then in the intercellular spaces between adjacent duct cells (Fig. 5 inset). The tracer was also present in the acinar lumina (Plate Fig. 8a) and subsequently in the intercellular spaces between most of the acinar cells (Plate Fig. 8b). Eventually the tracer reached the basal surfaces of the different cellular components (Plate Figs 5, 8 and 9) and was also seen in the basal infoldings of the granular and striated duct cells (Fig. 9). At this stage, a few endocytic vesicles containing reaction product were present in the cytoplasm of some striated and granular duct cells (Fig. 9 inset). In addition, some of the main excretory duct cells contained labelled endocytic vesicles.

At all later stages, luminal reaction product was found less frequently, whereas HRP was consistently present in the intercellular spaces. The amount of reaction product at the basal surfaces of the cells successively increased. Eventually, the tracer was found in the connective tissue, filling the spaces between the acini and ducts. Some macrophages had taken up the tracer and vacuoles in these cells occasionally stained for HRP. Nerve bundles, blood vessels and lymphatic vessels were surrounded by the tracer (Plate Figs 10 and 11). It was also seen in the intercellular spaces between the endothelial cells. Subsequently, reaction product was found in the lumina of blood and lymphatic vessels, presumably representing removal of the administered tracer.

Isotonic saline solution

As previously (Qvarnström *et al.*, 1981), the intraglandular pressure development during infusion of isotonic saline solution differed from that recorded during infusion of water-soluble contrast medium. About 15 s after start of infusion, the pressure began to increase, reaching approx. 45 mmHg (Fig. 2b). This was followed by about a 15 mmHg decrease to a plateau level. A small peak during the first pressure increase also occurred during infusion of isotonic saline solution. It was, however, less frequent and not quite as pronounced as that caused by infusion of the contrast medium.

Infusion of saline solution containing HRP caused the same type of morphological changes as did infusion of Renografin, although dilatation of the lumina was not as marked. As with infusion of contrast medium, the most severe changes were seen in the intercalated ducts; however, the desmosomes in these glands usually had a normal appearance.

The tracer first appeared in the lumina of the intralobular ducts and acini. The apical plasma mem-

branes of these cells became heavily stained. The luminal membranes of the main excretory duct cells were also lined with the tracer (Plate Fig. 12). Later, reaction product was found in the intercellular spaces and at the basal surfaces of the intralobular ducts and acini.

Some cellular uptake was also seen in these glands. Thus, endocytic vesicles were found in intralobular and main excretory duct cells. In addition, some duct cells with diffuse cytoplasmic reaction product were found in glands infused with isotonic saline solution. A large amount of the tracer was present in some intercalated duct cells showing abnormal cytoplasm and occasionally disrupted cell membranes (Plate Fig. 13). Cells with HRP in the cytoplasm were also found in the striated and main excretory ducts. In particular, the tuft cells (Hand, 1981) of the main excretory duct quite frequently contained tracer (Plate Fig. 14). At later stages, a considerable amount of reaction product was found in the connective tissue, and in the lumina of blood and lymphatic vessels.

DISCUSSION

The intraglandular pressure development recorded during infusion of water-soluble contrast medium or isotonic saline solution was essentially the same as previously (Qvarnström *et al.*, 1981). Adding the water-soluble HRP to the two solutions apparently did not change their physical or physiological properties significantly. However, the small peak seen during the first pressure increase did not occur in the earlier investigation. Subsequent experiments showed that prior stimulation with methacholine chloride (5 µg/kg intravenously) resulted in an even first pressure rise, suggesting that the abrupt peak was due to lack of secretory stimulation of the glands. Stimulation of secretion increases the permeability of the intercellular junctions to a retrogradely administered tracer (Oliver and Hand, 1978); thus, this initial peak could reflect resistance of the junctions to fluid outflow during infusion in the unstimulated gland. Insertion of the glass cannula into the duct evoked secretion in some cases, whereas in other glands no spontaneous salivary flow was noticed. Variations in occurrence and height of the initial peak therefore could be explained by differences in the extent of mechanical stimulation of the autonomic nerves supplying the gland.

Luminal dilatation of the intralobular ducts increased successively throughout infusion. Increased pressure of saliva contained in the gland most likely caused the earliest changes as dilatation of the ducts was seen in areas where no reaction product was present. It is known that secretion of saliva against a head of pressure causes ballooning disruption of striated ducts in the dog submandibular gland (Emmelin, Garrett and Gjørstrup, 1977). The morphological changes were seen in all three components of the intralobular duct system whereas the acinar lumina maintained their normal size throughout infusion with either medium. The apical junctional complexes between glandular cells in various segments are likely to resist a certain degree of pressure before allowing penetration of saliva and the infused media into the intercellular spaces. The different re-

sponses suggest that the junctional complexes of the submandibular acinar cells are more permeable than those of the duct cells. In cat sublingual gland, however, ruptures of acini are reported to occur at early times after duct ligation, while morphological changes in the duct system appear much later (Harrison and Garrett, 1972). At a high enough pressure desmosomes in the intercalated ducts became considerably widened, whereas in other parts of the gland no morphological changes were seen in these junctions. These variable results may also be explained by other differences in the physiological properties of the tissue, as well as possible differences in pressure at different parts of the luminal system during infusion. Despite the penetration of the tracer through the junctional complexes, no structural changes were detected in the tight junctions between either acinar or duct cells.

Reaction product was first seen in the lumina of the intralobular ducts and acini whereas, after the top of the first major peak, large amounts were present intercellularly and basal to most of the cells in the areas containing tracer. This would correspond to the ductal and so-called paranchymal filling characterizing the sialographic appearances seen in the two early phases of the pressure curve (Qvarnström *et al.*, 1981). Intercellular reaction product was more consistently found in the acini than in the ducts. However, all segments of the intralobular duct system contained tracer intercellularly at some stages of filling. This was seen whether or not a large amount of tracer was present basally, in contrast to the findings of Garrett and Parsons (1976). This suggests that penetration of infused material through the intercellular junctions of the three types of duct cells occurred, although the tracer probably also entered these intercellular spaces secondarily from the interstices, a pathway that has been reported after arterial administration of HRP (Garrett and Parsons, 1974).

The distribution and effects of isotonic saline solution were similar to those of water-soluble contrast medium although some differences were obvious. These are probably due to differences in the physical and physiological properties of the two media. Thus, after infusion of isotonic saline solution, the tracer bound to the luminal border of the ducts and acini, while binding was not apparent after infusion of the contrast medium. Binding of HRP to the luminal cell surface after retrograde injection has also been seen in rabbit and dog submandibular glands (Garrett and Parsons, 1976; Parsons and Garrett, 1977). The observed differences may be due to the difference in pH of the two media. With HRP added (isoelectric point 7.3–7.4; Rennke, Patel and Venkatachalam, 1978), the pH of saline solution and Renografin were 5.5 and 7.3, respectively. In the slightly acidic saline solution, the positively-charged HRP molecules would bind to the luminal membrane (Böck, 1972) whereas, dissolved in the contrast medium, the peroxidase would have less affinity for the cell surface. The reduced binding of HRP in the contrast medium may also be due to complexing of the peroxidase with constituents of the medium.

Diffuse cellular uptake of the tracer was not seen after administration of contrast medium, whereas in glands infused with isotonic saline solution the cyto-

plasm of occasional intralobular and main duct cells contained reaction product. As damage of the luminal membrane was seen in some of these cells, it is likely that the HRP gained access to the interior of the cell after membrane disruption. This could cause changes in the cytoplasm and the cellular organelles, as was seen in many of these cells.

The observed variations in intraglandular pressure are apparently dependent upon the distribution and effects of the infused media. Thus, distensibility of the luminal space, permeability of the junctional complexes, diffusion of the media through the connective tissue and its removal from the gland are likely to influence the intraglandular pressure. A direct correlation between the pressure development and the morphological appearance could not be made, however, as the degree of filling in different areas of the gland varied at all phases of the pressure curve. Large amounts of reaction product were seen basal to some cells at early times, whereas unfilled areas were occasionally found even at the plateau level. This indicates that a number of different events were occurring in the gland at all times during administration of the medium. Thus, the observed variations in pressure during infusion are probably not due to specific events, but reflect the sum of all structural alterations occurring at a particular time.

It is likely that the distribution and effects of water-soluble contrast medium during and after sialography in man are similar to those we observed. The optimal degree of filling of the duct system and the acini for most diagnostic purposes would correspond to that obtained at the latter part of the ascending phase of the first major pressure peak (Qvarnström *et al.*, 1981). The extent of morphological changes may differ, however, due to the supportive effects of the myoepithelial cells in the unanesthetized patient, which would reduce the distensibility of the luminal system (Emmelin *et al.*, 1977). Penetration of the contrast medium through the junctional complexes into the connective tissue and its uptake by blood and lymphatic vessels, as observed in the experimental system, probably contribute to the removal of the contrast medium from the gland following clinical sialography.

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Plate 1.

Fig. 3. Light micrograph of a rat submandibular gland showing normal appearance of striated (std) and granular (grd) ducts. Toluidine blue stain. 1 μ m section. $\times 372$

Fig. 4. Infusion of water-soluble contrast medium causes dilatation of the intralobular ducts. The luminal border of the granular ducts (grd) shows a scalloped appearance, whereas the striated ducts (std) increase more evenly in size. The lumina of the acini appear unaffected. Toluidine blue stain. 1 μ m section. $\times 372$

Fig. 5. Light micrograph showing an uneven distribution of contrast medium throughout the gland. Heavily stained areas (arrow) are next to areas where no HRP reaction product is present. Light-green counter stain. 2.5 μ m section of tissue incubated for HRP activity. $\times 70$. Inset: Reaction product is present in the intercellular spaces of the intralobular ducts. Tracer also surrounds the acini. $\times 233$

Plate 2.

Fig. 6. The lumen (L) of the intercalated duct (icd) is irregularly enlarged. The most severe changes are in the proximal part of the duct, next to the acini (ac). $\times 6225$. Inset: At a later stage showing a widening of the desmosomes (d) between the intercalated duct cells. $\times 18,000$

Fig. 7. The luminal border of the granular ducts shows a scalloped appearance with the apical junctional regions projecting into the lumen (arrows). Secretory granules in the cytoplasm appear compressed. $\times 4725$

Plate 3.

Fig. 8. (A) Reaction product fills the acinar lumen (L) but is not present in the junctional complexes or the intercellular spaces (arrow). $\times 34,780$ (B) At a later stage showing the tracer in the intercellular spaces (arrowheads) and at the basal surface (arrows) of the acinar cells. Note tracer in the junctional complex. Lumen (L). $\times 26,344$

Fig. 9. Basal infoldings of granular ducts containing tracer (arrows) in areas where there are large amounts basal to the cells. $\times 13,320$. Inset: Endocytic vesicles in the apical cytoplasm of a granular duct cell contain the tracer. $\times 33,300$

Plate 4.

Fig. 10. An arteriole (art) surrounded with HRP. Reaction product is seen between the smooth muscle and endothelial cells (arrow). A large amount of tracer (arrowheads) surrounds acini (ac) and nerve bundles (n). Reaction product is present in the intercellular spaces between acinar cells (open arrow). $\times 6800$

Fig. 11. Reaction product surrounds a lymphatic vessel (lv), intercalated duct (icd) and acini (ac). The tracer is also in the lumen of the lymphatic vessel. $\times 7395$

Plate 5.

Figs 12–14. Electron micrographs of the submandibular gland after infusion of saline solution containing HRP.

Fig. 12. Main excretory duct cell with reaction product lining the luminal membrane. Peroxisomes (arrow) are stained after incubation due to their catalase content. $\times 16,725$

Fig. 13. Damaged intercalated duct cell with cytoplasmic reaction product. $\times 10,500$

Fig. 14. Tuft cell of the main excretory duct with intracellular reaction product. Note prominent microvilli, numerous apical cytoplasmic vesicles and basal membranous complex (arrow). $\times 8250$

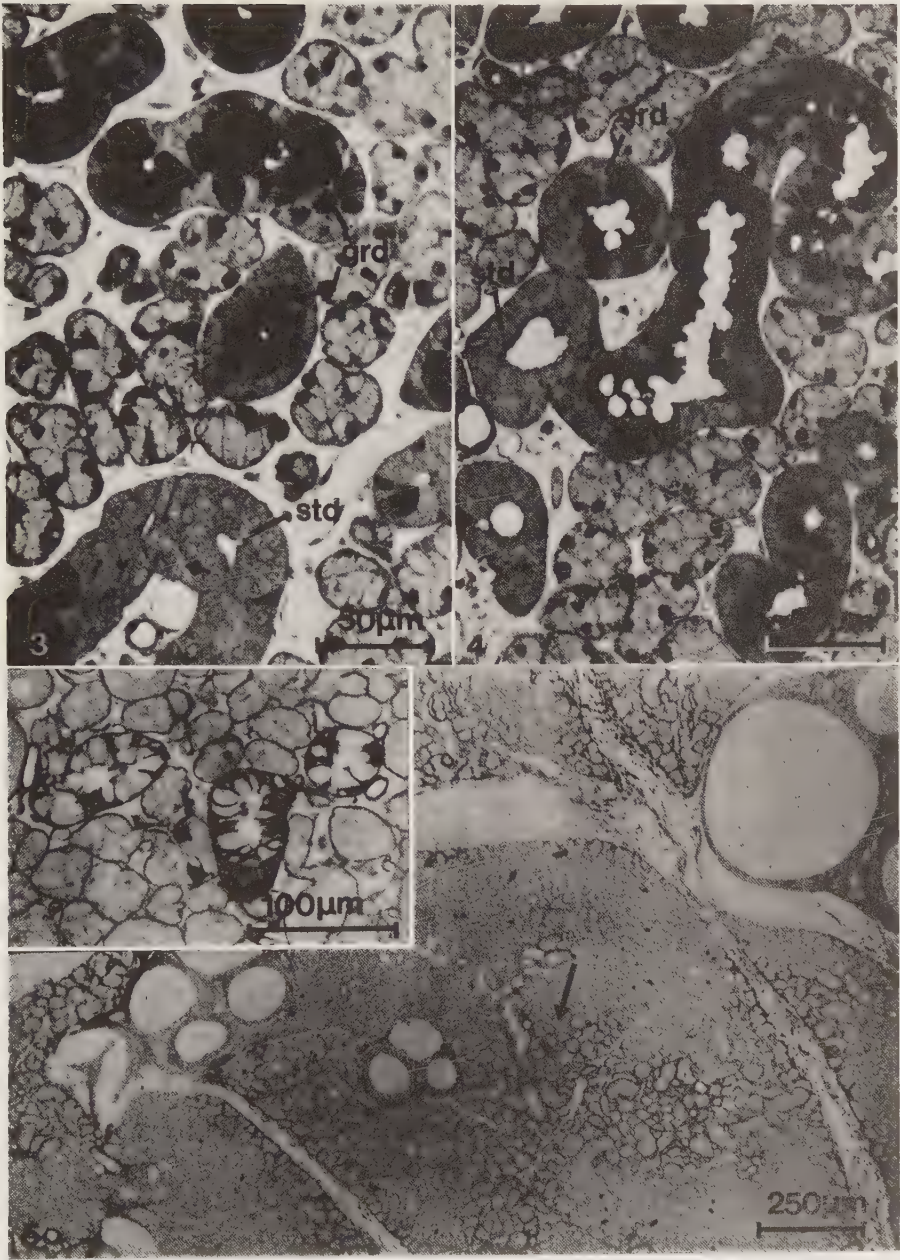


Plate 1.

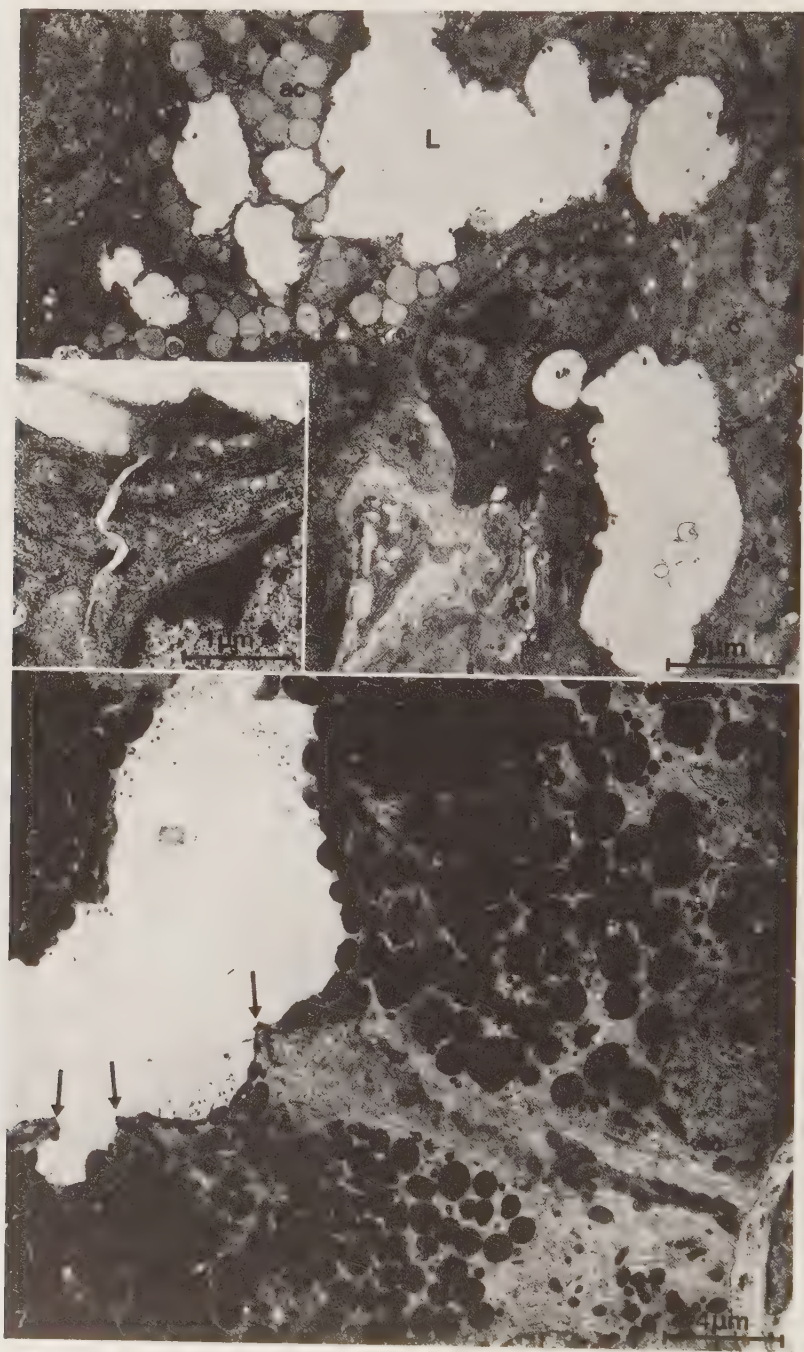


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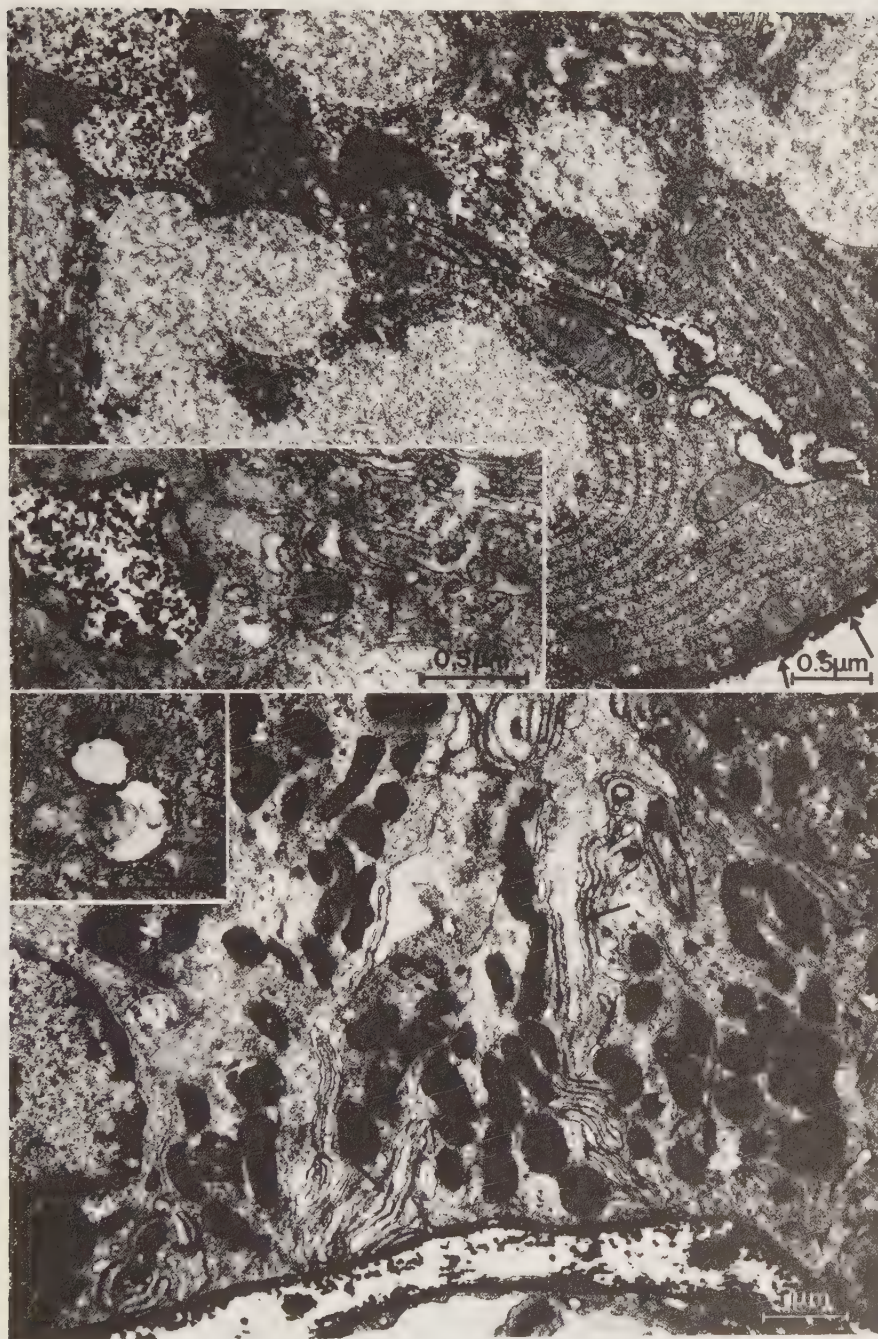
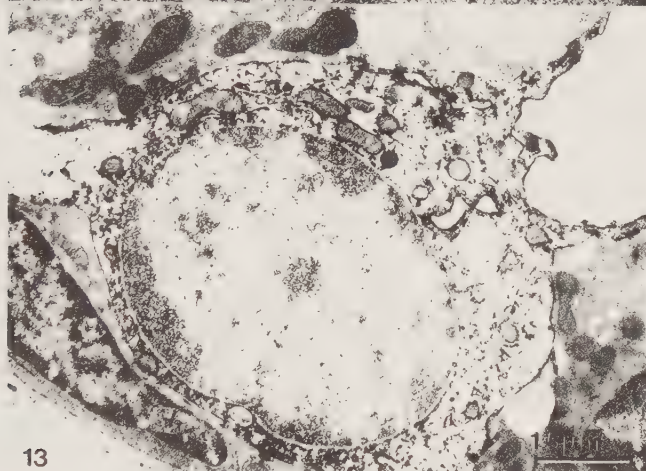
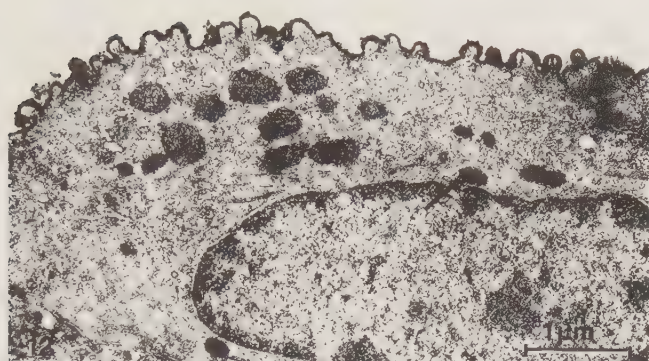


Plate 3.



Plate 4.



13



14

Plate 5.

A LIGHT AND ELECTRON MICROSCOPIC STUDY OF THE EFFECTS OF RETROGRADE INFUSION OF LIPID-SOLUBLE RADIOGRAPHIC CONTRAST MEDIUM INTO THE RAT SUBMANDIBULAR GLAND

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Summary—The intraglandular pressure was recorded during continuous retrograde infusion of lipid-soluble contrast medium, as the pressure developing in the gland has been shown to correlate with certain radiographic stages of glandular filling. Infusion was stopped at different points on the pressure curve and the gland was immediately fixed by vascular perfusion. The earliest detectable change, dilatation of the intralobular ducts, was most prominent in the intercalated duct segment. Later, cytoplasmic vacuoles in continuity with the luminal space were seen in the duct cells. Eventually the acinar lumina and intercellular canaliculi became widened. With continued infusion, a greater proportion of the duct and acinar lumina became dilated and the lumina increased successively in size. The most severe changes occurred in the intercalated duct segment and the acini. Due to an uneven distribution of the contrast medium, damaged areas were seen next to areas where the glandular components had a normal appearance. Consequently, the development of the intraglandular pressure could not be directly correlated with specific morphological alterations, but appeared to reflect all events occurring in the tissue at a particular time.

INTRODUCTION

Water-soluble and lipid-soluble radiographic contrast media are used for diagnostic purposes in radiographic examinations of various organs such as liver, kidneys and the gastrointestinal tract. They are also used in sialography, a radiographic examination of the salivary glands that comprises filling of the duct system and the acini retrogradely through the main excretory duct (Mason and Chisholm, 1975; Blair, 1976; Lowman and Cheng, 1976). Parameters related to the procedure, such as infusion pressure and rate of infusion, as well as the rate of removal of the contrast medium from the tissue, are likely to affect the degree of filling of the ductal system and the acini. To study systematically the relationship between these parameters and how they affect the appearance of the gland in the sialogram, an experimental model for sialography was developed (Qwarnström, Gjørstrup and Omnell, 1981). For each type of radiographic contrast medium, continuous infusion resulted in a characteristic intraglandular pressure development and different stages of glandular filling, as seen in the sialogram, could be correlated with different phases of the pressure curves obtained.

The effects of retrograde infusion of water-soluble contrast medium on the rat submandibular gland were previously studied by light and electron microscopy (Qwarnström and Hand, 1982). The morphological changes caused by the procedure consisted primarily of dilatation of the intralobular duct system. The differences in physical properties between the water-soluble and lipid-soluble contrast media are known to affect the interpretation of the sialogram (Goebel, 1977) and can also account for the differences in the pressure developing in the gland during

infusion of the two types of media (Qwarnström *et al.*, 1981). The aim of the present investigation was to determine the structural bases for the development of the intraglandular pressure during infusion and to study the effects on the tissue of the sialographic procedure using the lipid-soluble contrast medium.

MATERIALS AND METHODS

A total of 20 Sprague-Dawley rats weighing between 200 and 450 g were used. General anaesthesia was obtained by intraperitoneal injection of chloral hydrate (400 mg/kg body wt). After tracheotomy, the left submandibular gland excretory duct was dissected extraorally and cannulated approx. 5 mm anterior to the hilus using a saline-filled glass cannula (outside dia. = 0.5 mm) according to Ohlin (1965).

The intraglandular pressure was recorded using a pressure-monitoring system similar to that employed previously (Qwarnström *et al.*, 1981; see Fig. 1 in Qwarnström and Hand, 1982) and operated according to Emmelin, Garrett and Ohlin (1968). The glass cannula was connected to an infusion pump (Sage Instruments, syringe pump model 341A) and to a displacement transducer (Bentley Trantec, Model 800, No. 10118) which was connected to an amplifier and a recorder (LKB 2210). The syringe fitted to the pump and the polyethylene tubing connecting it with the gland were filled with lipid-soluble radiographic contrast medium, Lipiodol UF (Guerbet; iodine content 480 mg/ml, viscosity 25 mPa·s at 36°C). Other parts of the system were filled with isotonic saline solution (0.9 per cent). The system was calibrated with a pressure bottle and a Hg manometer connected to the transducer.

The radiographic contrast medium was infused through the main excretory duct at an infusion rate of 0.008 ml/min, as used in the previous studies. Before starting the infusion, the thoracic cavity was opened and the rat artificially respired with 95 per cent O₂ 5 per cent CO₂ during the experiment. The infusion was stopped at different degrees of filling as determined by the development of the intraglandular pressure (Text Fig. 1). The gland was immediately fixed by vascular perfusion through the ascending aorta with a modified Karnovsky (1965) fixative containing 2 per cent glutaraldehyde and 2 per cent formaldehyde (Ladd Research Industries) in 0.1 M cacodylate buffer, pH 7.4, with 0.05 per cent calcium chloride. Both sub-mandibular glands, the experimental and the contralateral used as a control, were removed and immersed in fresh fixative for approx. 3 h.

The parenchymal tissue and the proximal part of the main excretory duct were cut in 1 mm³ pieces, rinsed in sucrose buffer (0.1 M sodium cacodylate and 7 per cent sucrose, pH 7.4), and stored overnight at 4°C. After post-fixation for 2 h at room temperature in 1 per cent osmium tetroxide in sucrose buffer, the tissue was rinsed and stained *en bloc* for 2 h with 0.5 per cent uranyl acetate in distilled water containing 5 per cent sucrose. Dehydration through a graded series of ethanols preceded embedding in Spurr (1969) resin. In addition, a thin transverse slice from the centre of each gland was embedded in JB-4 plastic (Polysciences), sectioned and stained with haematoxylin-eosin or periodic acid-Schiff.

Thick (1 µm) sections of Spurr-embedded tissue, stained with toluidine blue, were examined by light microscopy. Thin sections of selected areas were cut with a diamond knife and mounted on bare copper grids. The sections were stained with uranyl acetate and lead citrate and examined in a Zeiss EM-10A electron microscope.

RESULTS

Infusion of lipid-soluble contrast medium caused an increase in intraglandular pressure, beginning about 5 s after the start of infusion (Fig. 1). After reaching about 40 mm Hg, the pressure increased more rapidly to about 100 mm Hg where it levelled off, forming a plateau. A second increase to a peak of approx. 150 mm Hg was followed by a decrease in pressure to about 115 mm Hg, where it then remained despite continual infusion. The intraglandular pressure development was highly reproducible, making it possible to ascertain that a correct infusion was achieved in the different experiments.

The earliest morphologic change after infusion of lipid-soluble contrast medium was dilatation of the intralobular ducts (Plate Figs 2 and 3), initially seen during the early parts of the first pressure increase. All three components of the intralobular duct system, i.e. intercalated, granular and striated ducts, were affected, each presenting a characteristic appearance. The lumina of the intercalated ducts were irregularly enlarged (Plate Fig. 4). The duct cells became flattened and compression of the cytoplasmic constituents occurred. The most severe changes were seen in the proximal part of this segment, next to the acinar cells. The luminal border of the granular ducts

presented a scalloped appearance (Fig. 3) with the apical junctional regions projecting into the lumen. The luminal expansion resulted in a compression of the apical granules. The striated duct lumina increased evenly in size with a minimal amount of scalloping. This segment was the least affected and flattening of the duct cells rarely occurred. The main excretory duct seemed unaffected by the infusion, showing a normal appearance at all times.

As infusion continued, the changes successively became more pronounced and additional morphological alterations were seen in some duct segments at later stages. The intercalated duct lumina appeared more irregular and considerable flattening of the duct cells occurred. Widening of the desmosomes between the intercalated duct cells was fairly common, especially in the proximal part of the segment (Plate Fig. 5). In the granular and striated ducts, the scalloped appearance of the luminal border was less pronounced (Plate Fig. 6). The apical microvilli of these cells were bent towards the cell surface, and at later times, were frequently seen parallel to and in contact with the luminal membrane of the cells (Fig. 6).

In glands where filling was continued until the developing pressure had reached the main peak, cytoplasmic vacuoles were frequently present in the different intralobular duct cells, mostly in the intercalated ducts (Plate Fig. 7). The vacuoles contained apparent secretory product and were occasionally continuous with the duct lumen. Their size varied from 0.1 to 10 µm, the larger ones frequently in proximity to a distorted, crescent-shaped nucleus.

Dilatation of the acinar lumina (Plate Fig. 8) was initially seen when the pressure reached the first plateau level. The intercellular canaliculi, luminal processes extending basally between adjacent acinar cells, also became widened, resulting in frequent profiles of branching lumina. This appearance successively became more pronounced until the main peak was reached, after which, in most cases, the individual canaliculi no longer could be distinguished.

The dilated lumina of the ducts and acini were often only partially filled with secretory product (Plate Fig. 9). A distinct electron-dense interface was usually present, separating the secretory material from

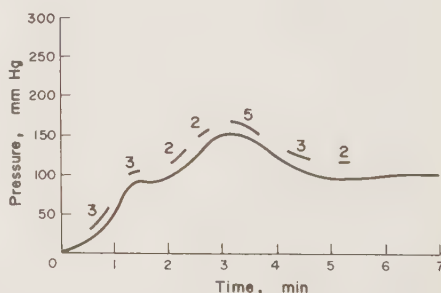


Fig. 1. Typical intraglandular pressure development during infusion of lipid-soluble contrast medium, Lipiodol UF. Lines parallel to the curve and corresponding numbers indicate where infusion was stopped and number of animals in the different experimental series.

an apparently empty part of the lumen (Plate Figs 9 and 11). Release of the secretory granule content into dilated acinar lumina was occasionally seen. Apparent secretory product was also found in the intercellular spaces (Plate Fig. 10), presumably due to a discharge of granule content at the lateral plasma membrane basal to the junctional complexes. In some cases, this lateral discharge seemed to cause compression of the acinar cells and deformation of the surrounding myoepithelial cells.

Continuous infusion caused extensive changes, particularly in the intercalated duct segment and in the acini (Plate Fig. 11). Dilatation of the lumina and a successive increase in number and size of the cytoplasmic vacuoles throughout infusion led to a pronounced compression of the intercalated duct cells. In the acini, continued increase in luminal size and widening of the canaliculi eventually resulted in marked deformation of the acinar cells and considerable compression of the cellular organelles. Occasionally, formation of cytoplasmic vacuoles was also seen in the acini. At this stage, the interlobular connective tissue spaces appeared widened in some areas.

Light microscopy revealed great variations in the morphological appearance in different parts of the gland (Plate Fig. 12). Areas with moderate to extensive damage were seen next to areas where ducts and acini had a normal appearance. The damage successively became more severe and a greater proportion of the tissue showed morphological changes as infusion continued. However, areas with different degrees of damage were seen in the tissue at all stages of glandular filling.

DISCUSSION

The pressure curves obtained exhibited a remarkably consistent shape. They varied, however, from the intraglandular pressure development recorded in the earlier investigation using Lipiodol UF (Qvarnström *et al.*, 1981). The difference was seen in the latter part of the curve, i.e. at stages when the gland was already filled with contrast medium. Thus, after reaching approx. 150 mm Hg, a decrease to a plateau level followed, rather than a third pressure increase, as noted in the study quoted. Additional experiments (not reported here) suggest that the observed difference is due to the use of different strains of rats in the two studies.

The earliest morphological changes were similar to those seen after infusion of water-soluble contrast medium or isotonic saline solution (Qvarnström and Hand, 1982). During infusion of these two substances with horseradish peroxidase (HRP) added as a tracer, dilatation of the intralobular ducts was seen prior to detection of HRP in the tissue. Thus, it was assumed that the initial changes were caused by pressure of the saliva present in the gland. This phenomenon most likely accounts for the changes seen at early stages during infusion of lipid-soluble contrast medium. Morphological alterations due to increased pressure in the tissue by saliva alone were reported by Emmelin, Garrett and Gjørstrup (1977), who found ballooning-disruption of striated ducts in dog submandibular gland after secretion against a head of pressure.

The morphological alterations seen at later stages were more severe than those observed even after prolonged infusion of water-soluble contrast medium or isotonic saline solution. This is probably due to the 3–4-fold higher intraluminal pressure induced during administration of the lipid-soluble medium. The difference in pressure can probably be explained by differences in physical properties of the two media. Thus, due to its high viscosity and low solubility, the lipid-soluble medium probably remains in the luminal space and leakage through the tight junctions during infusion is probably very limited. This was also observed in the radiographic study (Qvarnström *et al.*, 1981) where the ductal system of glands infused with lipid-soluble contrast medium was fairly well-defined in the sialogram after prolonged infusion. In contrast, a loss of the gland contour and a diffuse radiopacity were present at later stages using the water-soluble medium, which in the previous morphological study leaked into the intercellular spaces (Qvarnström and Hand, 1982).

The loss of the scalloped appearance in the striated and granular ducts as well as the observed compression of the microvilli towards the apical membrane seen at later times are probably direct results of the increased luminal pressure. Cytoplasmic vacuoles were most frequently present in the intercalated duct segment. We considered the vacuoles to be extensions of the lumen as they occasionally contained apparent secretory product and continuity with the luminal space was seen in some sections. It is possible that increased pressure on the luminal surface causes an increased rate of fusion and release of the duct granules. The addition of granule membrane to the luminal surface may thus result in formation of vacuoles in continuity with the luminal space, partly or totally filled with contrast medium. A similar explanation is less likely for the formation of the vacuoles in the striated ducts, as their granule content is considerably less than that of granular or intercalated ducts and, in most cases, these vacuoles were more basally located.

The higher luminal pressure probably also accounts for the dilatation of the acinar lumina, not seen in the previous study (Qvarnström and Hand, 1982). Discharge of acinar secretory granules into the lumen and intercellular spaces was noticed at later times. It appeared to be related to compression of the acinar cells caused by the continued luminal expansion, probably resulting in increased apposition of the secretory granules to the cell membrane. Granule discharge may also be due to receptor stimulation caused by release of endogenous neurotransmitters or to pressure-induced changes in membrane permeability resulting in alterations of intracellular $[Ca^{2+}]$ or other second messengers. Lateral discharge of secretory granules has also been observed in rat parotid gland after prostaglandin administration (Lillie, 1974).

The lipid-soluble contrast medium was lost during processing of the tissue for microscopy. The empty areas seen in some of the lumina probably represent the space previously occupied by the medium, because the infused lipid-based material is likely to displace rather than mix with the saliva present in the gland (Goebel, 1977). The electron density of the

interface may be explained by an increased concentration of the salivary content at this site, due to the amphipathic nature of the secretory proteins (Mac Ritchie, 1978).

The differences in the degree of damage seen in the various glandular segments could be due to differences in susceptibility to the induced pressure. The observations may also be explained by variations in the pressure exerted in different parts of the excretory system. At all stages of filling, markedly damaged areas were seen next to areas where the tissue had a normal appearance, indicating that retrograde infusion results in an uneven distribution of the lipid-soluble contrast medium. This phenomenon was demonstrated directly for water-soluble contrast medium by the use of HRP as an electron-dense tracer (Qwarnström and Hand, 1982). Thus, the variations in intraglandular pressure development cannot be correlated to specific morphological changes, but reflect all alterations occurring in the tissue at a given time.

The morphological alterations appear at stages of filling used for diagnostic purposes (Qwarnström *et al.*, 1981). Similar changes may occur in the glandular tissue during clinical sialography using lipid-soluble contrast medium, although the supportive effect of the myoepithelial cells in the unanaesthetized subject, resulting in reduced distensibility of the luminal system (Emmelin *et al.*, 1977), may influence the pattern of morphological alterations. Severe damage of fairly large areas, seen in the intercalated duct segment and acini, may also be apparent in the sialogram. The appearance of sialectasis in dog parotid gland can be due to damage of the intralobular ducts induced during the sialographic procedure (Garrett *et al.*, 1976). It thus seems likely that changes in the structure of the glandular tissue induced by infusion of lipid-soluble contrast medium result in an altered sialographic appearance of the gland.

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Plates 1–4 overleaf.

Plate 1.

Fig. 2. Light micrograph of control submandibular gland showing normal appearance of the various glandular components, granular duct (grd), intercalated duct (icd) and acini (ac). Haematoxylin-eosin stain, 2.5 μ m section. $\times 304$

Fig. 3. Light micrograph of submandibular gland infused with lipid-soluble contrast medium for about 50 s, showing the initial change of dilatation of the intralobular ducts. The granular ducts (grd) show a scalloped appearance whereas the intercalated ducts (icd) are irregularly enlarged. The acini (ac) appear unaffected. Haematoxylin-eosin stain, 2.5 μ m section. $\times 318$

Figs 4-11. Electron micrographs of the rat submandibular gland after infusion of lipid-soluble contrast material.

Fig. 4. The lumen of the intercalated duct (icd) is irregularly enlarged. The most severe changes are in the proximal part, next to the acini (ac). In a number of the duct cells, cytoplasmic vacuoles (vac) are present, some of which are in continuity with the luminal space (L). $\times 5328$

Fig. 5. After further infusion, widening of the desmosomes (d) between adjacent duct cells is seen, most commonly in the intercalated duct segment. $\times 46,028$

Plate 2.

Fig. 6. A later stage; the scalloped appearance of the luminal border of the granular and striated ducts is less pronounced. The microvilli (mv) at the luminal border are bent towards the cell surface (arrows) and in contact with the apical membrane (arrowheads) $\times 7770$

Fig. 7. Cytoplasmic vacuoles (vac) of variable size are most frequently present in the intercalated ducts. The larger ones are in proximity to a compressed, crescent-shaped nucleus (n). Continuity with the lumen (L) is occasionally seen (arrow). $\times 8880$

Plate 3.

Fig. 8. A later stage; the lumina of the acini are dilated and the intercellular canaliculi (ca) widened, resulting in the appearance of branching lumina (L). $\times 6384$

Fig. 9. The salivary content is often confined to one part of the luminal space, the rest being apparently empty. An electron-dense interface is present (arrows). Proximal part of intercalated ducts. $\times 5548$

Fig. 10. Discharge of secretory product (arrows) at the lateral cell membrane basal to the junctional complexes between acinar cells (ac). The secretory material is retained in the widened intercellular spaces by the basement membrane (bm). Myoepithelial cell process (my). $\times 14,972$

Plate 4.

Fig. 11. A later stage still; severe changes are present, particularly in the intercalated duct segment (icd) and the acini (ac). There is considerable compression of these cells due to dilatation of the lumina and intercellular canaliculi, and an increase in number and size of cytoplasmic vacuoles. Note the dense material in the vacuoles of the intercalated duct and acinar cells (arrows). Arteriole (art). $\times 3780$

Fig. 12. Light micrograph of submandibular gland infused with lipid-soluble contrast material showing areas with different degrees of damage (arrows) next to areas where the tissue has a nearly normal appearance (arrowheads). Haematoxylin-eosin stain, 2.5 μ m section. $\times 361$

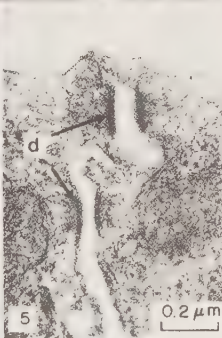
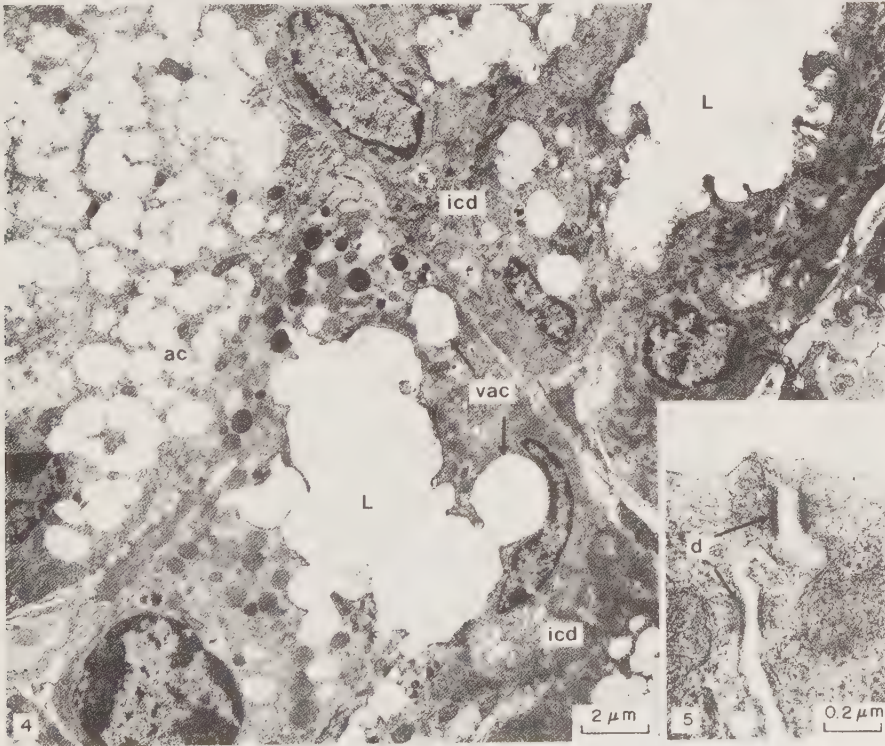
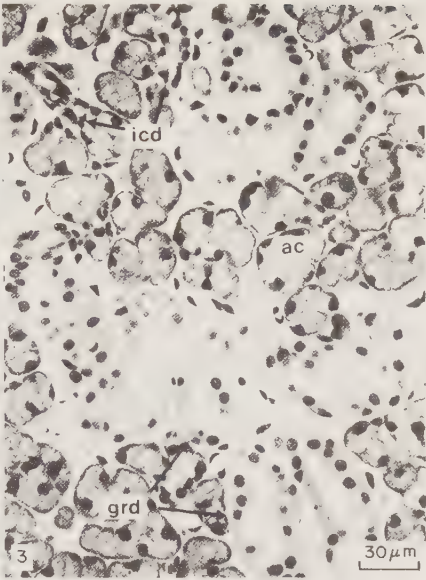
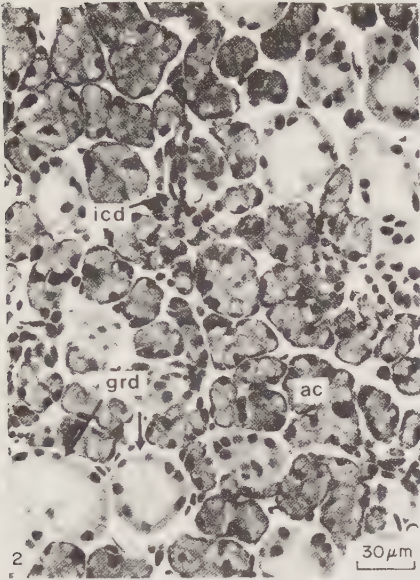


Plate 1.

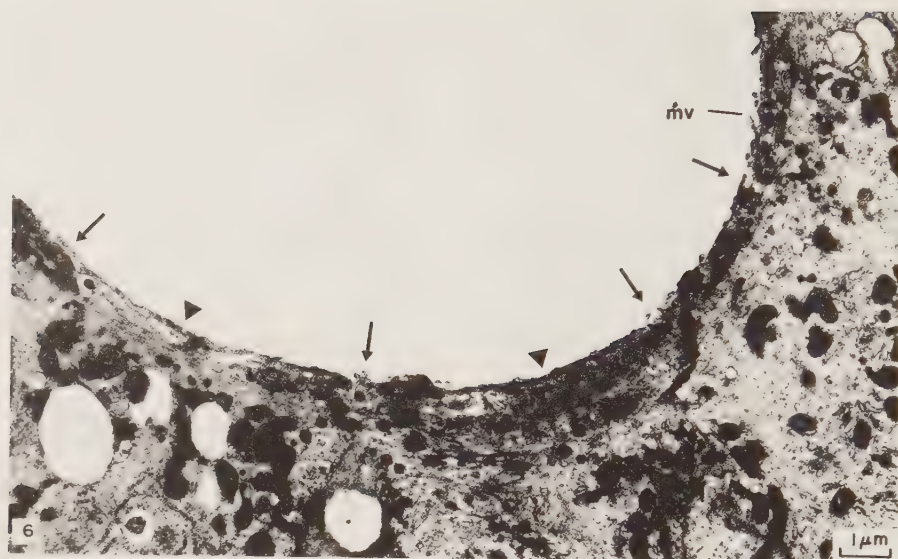


Plate 2.

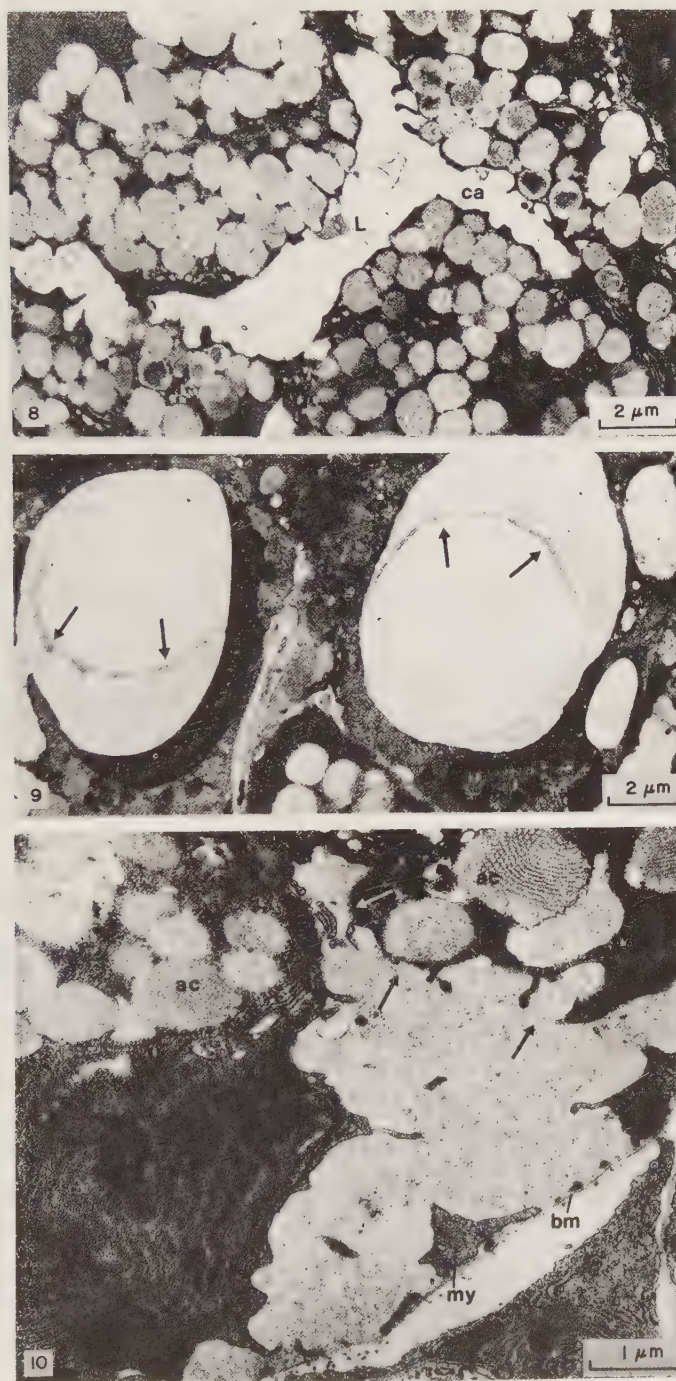


Plate 3.

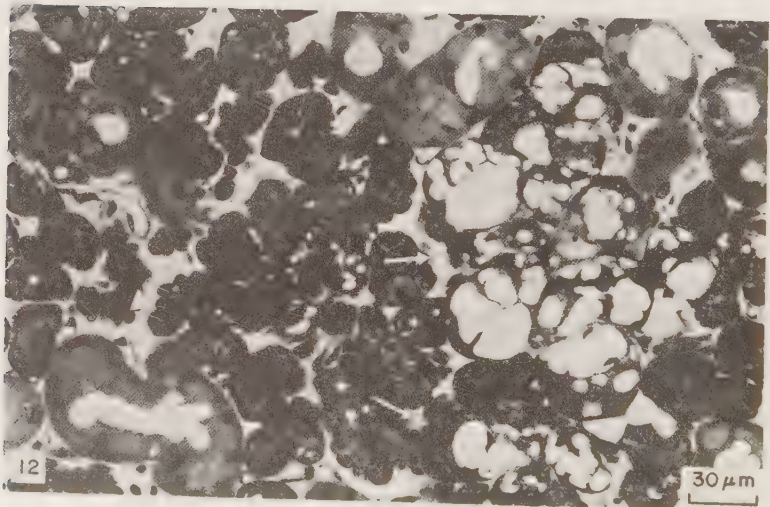
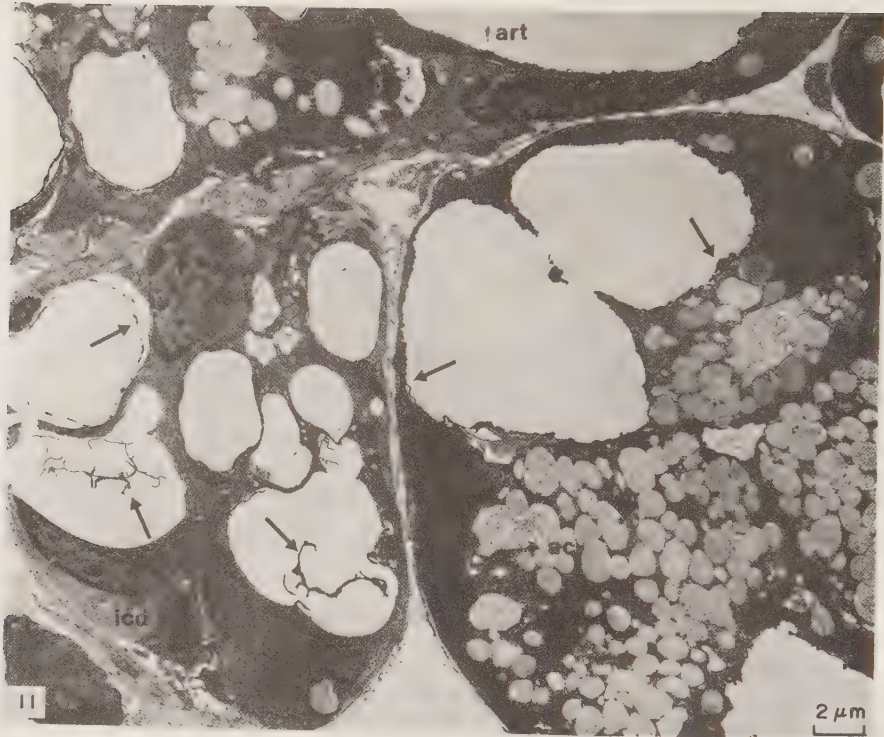


Plate 4

A technique for intraoral cannulation and infusion of the rat submandibular gland

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KEY WORDS: *Rats; submandibular gland*

The paper describes a technique for intraoral cannulation of the rat submandibular gland. The technique which avoids surgical intervention and causes a minimum of tissue damage allows for long term studies of both submandibular glands in the same animal. The method has been used for saliva collection and retrograde infusion of radiographic contrast medium. Normal function and structure of the gland are preserved after the cannulation procedure.

In studies on the rat submandibular gland, both extra- and intraoral cannulation of the main excretory duct have been used. Extraoral cannulation is routinely employed in investigations comprising retrograde infusion (4–6) and other studies involving induction of pressure into the luminal system of the gland (7, 8). The preparation technique used to expose the main excretory duct necessitates sacrifice of the animals after the experiment. Intraoral cannulation allows survival of the animals, but has been used primarily for collection of saliva (1, 2, 9).

The present paper describes a technique for intraoral cannulation which avoids surgical intervention and causes a minimum of tissue damage. The procedure allows for long term studies and can be used for both saliva collection and retrograde infusion through the natural orifices of the submandibular gland.

MATERIAL AND METHODS

Sprague-Dawley male rats (250–500 g) were anesthetized by intraperitoneal injection of sodium pentobarbital (45 mg/kg bw). The animals were placed prone on a board with their head

lifted and supported by a horizontal metal wire running behind the upper incisors, approximately 75 mm above the board. The lower jaw was held down with a silk ligature, placed behind the lower incisors and secured on the board. Another silk ligature was placed through the tip of the tongue so that it could be pulled upwards and aside, thus providing access to the orifices of the submandibular glands (Fig. 1).

The orifices, located just lateral to the medial tip of the sublingual caruncle, were observed through a stereo microscope ($\times 30$). Polyethylene tubing (Intramedic 7410, Clay Adams), which had been pulled to a fine solid tip over a laboratory hot plate, was introduced into the main excretory duct of the submandibular gland. The outer 4–5 mm of the duct was dilated with the tubing. Next, a PE 7410 catheter pulled to an outer diameter of approximately 0.5 mm was inserted into the dilated portion of the duct. To prevent leakage, the soft tissue was allowed to dry and adhere to the tubing before the catheter was pulled slightly anteriorly and medially in order to stretch the caruncle and the outer portion of the duct (Fig. 1). The tubing was positioned along the distal surface of the lower incisor on the contralateral side, at the level of the incisal edge.

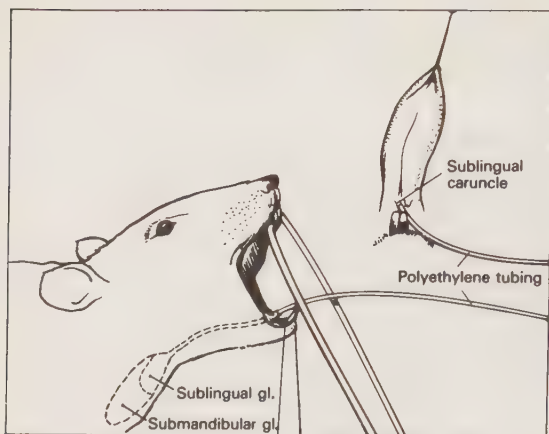


Fig. 1. Schematic drawing showing head position of the rat and direction of tubing after cannulation of submandibular gland. Polyethylene tubing is inserted into the main excretory duct. The upper jaw rests on a metal wire and the lower jaw is held down with a silk ligature, placed around the lower incisors. Inset: The tongue is pulled upwards and aside providing access to the orifices, located on the sublingual caruncles. The tubing is placed on the contralateral side, at the level of the incisal edge.

During the experiments, the respiratory tract was kept free of secretions from the other salivary glands by suctioning of the pharynx with a polyethylene tubing connected to a syringe or to a water pump aspirator.

RESULTS AND DISCUSSION

This intraoral cannulation procedure was applied to pressure monitored infusion using equipment similar to that employed earlier (4). The system was used for retrograde infusion of both water- and lipid-soluble radiographic contrast media (Fig. 2). Infusion of the water soluble material resulted in a peak pressure of about 50 mm Hg. During administration of the lipid based compound, the induced pressure was considerably higher, reaching a peak of approximately 150 mm Hg. To avoid leakage between the catheter and the walls of the duct at higher pressures, a cotton ligature was tied around the stretched papilla once the cannula was inserted.

This cannulation procedure has also been used in studies involving collection of stimulated



Fig. 2. Profile radiograph showing the main excretory duct containing lipid soluble radiographic contrast medium (arrowheads) and overfilled submandibular gland (arrow). The tubing inserted into the orifice (small arrow) also contains contrast medium.

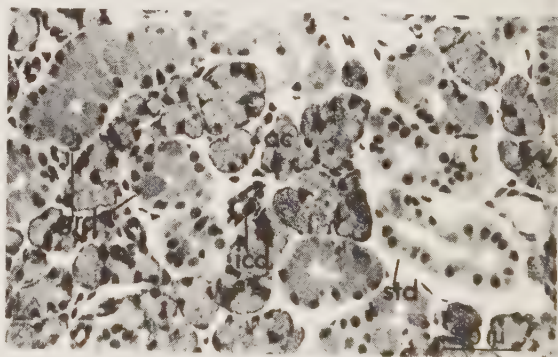


Fig. 3. Light micrograph of submandibular gland after cannulation of the main excretory duct. Both intralobular ducts and acini (ac) have a normal appearance. Striated duct, (std), granular duct (grd) and intercalated duct (icd). Periodic acid-Schiff-Hematoxylin stain $\times 210$.

(pilocarpine-HCl; s.c., 2.5 mg/kg bw) saliva from both glands on three consecutive weeks (3). Analyses of flow rate, protein content and levels of Na⁺ and K⁺ revealed no significant changes in the composition of saliva from week to week, suggesting that the cannulation procedure does not alter the function of the gland.

Submandibular glands from three animals, sacrificed 10 hrs, 30 hrs and 4 days respectively, after the cannulation procedure, were examined by light and electron microscopy. The morphology of the gland was normal at all times (Fig. 3). No difference could be detected between the experimental gland and the contralateral that served as control. Cross sections through the cannulated orifices showed slight stretching of the lining epithelial cells, whereas the surrounding tissue had normal appearance.

CONCLUSION

Long term studies of the rat submandibular gland can be made, using the described procedure for intraoral cannulation. The method allows for several collections of saliva from both contralateral glands of the same animal. In addition, retrograde infusion of foreign substances through the natural orifice of the gland is possible and induced pressures of up to 250 mm Hg may be tolerated. The technique excludes surgical intervention and both normal function and structure of the gland are preserved after the procedure.

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A morphologic study of the recovery of the rat submandibular gland after retrograde infusion

I. Water-soluble radiographic contrast medium

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The recovery of the rat submandibular gland after retrograde infusion of water-soluble radiographic contrast medium was studied using an experimental model. During continuous monitoring of the developing intraglandular pressure, the glands were subjected to ductal and slight parenchymal filling or heavy parenchymal filling with the medium. The animals were killed after varying recovery periods, and the tissue was prepared for light and electron microscopic examination. Dilation of the ductal lumina, induced during ductal and slight parenchymal filling, was successively reduced and, generally, the parenchyma had a normal appearance at 30 h. In glands subjected to heavy parenchymal filling, the changes in the intralobular ducts were more pronounced and were also seen at later times after infusion. Alterations in the acini, comprising fusion of secretory granules, vacuole formation and dilation of the acinar lumina and intercellular canaliculi, were observed. At later times, atrophy of the parenchymal cells occurred together with an apparent proliferation of the connective-tissue stroma, as well as an increase in the number of small blood vessels. An inflammatory cell-infiltrate was seen in both groups of animals, but was most prominent in glands subjected to heavy parenchymal filling. The infiltrate, comprised primarily of macrophages and polymorphonuclear leukocytes, reached a peak at 20 h after infusion. At later times, mast cells and occasional eosinophils were seen. The observed alterations and the pattern of recovery are most likely due to the induced intraglandular pressure and the following inflammatory reaction. It is also possible that the changes, to some extent, are influenced by the presence of the contrast medium in the tissue.

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Sialography is a radiographic examination of the salivary glands after filling of the luminal space with contrast medium. Ductal filling is achieved when the main excretory duct and the intralobular ducts are visualized in the sialogram. Further infusion results in paren-

chymal filling, where the acinar lumina are also believed to be filled (Samuel 1950, Ollerenshaw & Rose 1951, Lowman & Cheng 1976).

The effect of retrograde infusion of radiographic contrast medium on the tissue

was studied by light and electron microscopy using the rat submandibular gland as a model (Qwarnstrom & Hand 1982). Different degrees of filling of the gland were determined by correlation with the developing intraglandular pressure (Qwarnstrom et al. 1981). It was found that retrograde administration resulted in an uneven distribution of water-soluble radiographic contrast medium throughout the tissue, and that the morphological changes induced by the procedure varied to some extent in different parts of the gland at all stages of filling. These alterations, primarily consisting of luminal dilation, were seen in all three segments of the intralobular duct system. The initial changes occurred at lesser degrees of filling than those used in clinical sialography. The changes became successively more pronounced, and a larger proportion of the tissue was affected by increased filling.

The present investigation was carried out in order to study the recovery of the gland after different degrees of filling with water-soluble radiographic contrast medium and to characterize the long-term effects of the sialographic procedure.

Material and methods

A total of 33 Sprague-Dawley rats, weighing between 225 and 500 g, were used. General anesthesia was obtained by intraperitoneal injection of sodium pentobarbital (45 mg/kg b.w.). After dilation of the orifice, the left submandibular duct was cannulated intra-orally with fine polyethylene tubing as described by Omnell and Qwarnstrom (1983).

The polyethylene tubing was connected to an infusion pump (Sage Instruments, syringe pump Model 341 A) and a pressure monitoring system, the same as that used previously (see Fig. 1 in Qwarnstrom & Hand 1982), and operated according to Emmelin et al. (1968). The pressure recording device consisted of a displacement transducer (Statham 21207p 23BB) connected to an amplifier and recorder (Hewlett-Packard 321). The syringe fitted to the pump and the polyethylene tubing connecting it to the gland were filled with water-soluble radiographic contrast medium, Renografin 60% (Squibb, iodine content 292 mg/ml, viscosity 4 mPa·s at 36°C). Other parts of the system were filled with distilled water. The system was calibrated with a pressure

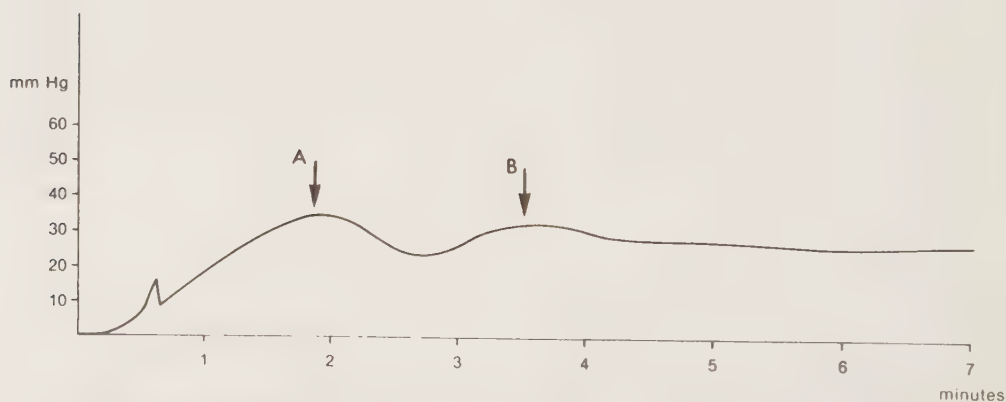


Fig. 1. Typical intraglandular pressure development during retrograde infusion of water-soluble radiographic contrast medium. The arrows indicate where infusion was stopped to achieve ductal and slight parenchymal filling (A), and heavy parenchymal filling (B), respectively.

bottle and a mercury manometer connected to the transducer. The development of the intraglandular pressure during retrograde infusion (0.008 ml/min) using an intraoral cannulation technique was the same as that recorded previously after extraoral cannulation (Qwarnstrom & Hand 1982), and could thus be used to ascertain a correct infusion, and to determine the glandular filling (Qwarnstrom et al. 1981). In 16 animals, infusion was discontinued when a ductal and slight parenchymal filling was achieved. The left glands in 17 animals were subjected to heavy parenchymal filling (Fig. 1) (Stage C, Qwarnstrom et al. 1981).

During the first two days after infusion, the rats were given ground laboratory chow (Purina), after which they were fed their normal diet of pelleted chow. At different times after infusion (10, 20 and 30 h, and 4, 7 and 10 days) 2–5 animals from each group were killed. The animals were anesthetized with chloral hydrate (400 mg/kg b.w.) and the submandibular glands were fixed by vascular perfusion through the ascending aorta using a modified Karnovsky's (1965) fixative containing 2% glutaraldehyde (Ladd Research Industries) and 2% formaldehyde (Polysciences Inc.) in 0.1 M sodium cacodylate buffer, pH 7.4, with 0.05% calcium chloride. Both submandibular glands, the experimental and the contralateral used as a control, were removed and immersed in fresh fixative for approximately 3 h.

The glandular tissue and the proximal part of the main excretory duct were cut in 1 mm³ pieces, rinsed in 0.1 M cacodylate buffer with 7% sucrose (pH 7.4) and stored overnight at 4°C. After postfixation for 2 h at room temperature in 1% osmium tetroxide in the same buffer, the tissue was rinsed and stained *en bloc* for 2 h with 0.5% uranyl acetate in distilled water containing 5% sucrose. Dehydration through a graded series of ethanols preceded embedding in Spurr's (1969) resin.

Thick (1 µm) sections stained with toluidine blue were examined by light microscopy. Thin sections of selected areas were cut with a diamond knife and mounted on bare copper grids. The sections were stained with uranyl acetate and lead citrate and examined in a Zeiss EM-10A electron microscope.

In addition, a thin transverse slice from each gland was embedded in JB-4 plastic (Polysciences, Inc.), sectioned (2.5 µm) and stained with hematoxylin-eosin, periodic acid-Schiff-hematoxylin or Giemsa and examined by light microscopy.

Results

The types of morphological alterations observed in the glandular tissue 10 h after infusion of water-soluble contrast medium were similar to those seen immediately following the procedure (Qwarnstrom & Hand 1982). They consisted primarily of luminal dilation of the intralobular ducts (Figs. 2, 3) with an irregular or scalloped appearance of the luminal border. The severity of the alterations varied to some extent between glands subjected to the same degree of filling, and between different parts of the same gland. In general, the changes were more common and more pronounced in the overfilled glands than in those where infusion was stopped when ductal filling was achieved. The appearance of the glands gradually returned to normal; however, a number of changes not present immediately following the procedure were noted at later times.

In glands subjected to ductal filling, the luminal diameter of the ducts had largely returned to normal by 20 h. However, the irregularity of the intercalated duct lumen and the scalloped appearance of the luminal border of the granular and striated ducts were still apparent at 30 h after infusion. In the intercalated ducts, sections of the irregular luminal surface frequently gave the appearance of

vacuoles in the apical cytoplasm (Fig. 4). Although both striated and granular ducts had a scalloped luminal border, this was most prominent in the granular ducts where compression of the secretory granules occurred, and apparent membrane-bound vacuoles were present in the supranuclear cytoplasm (Fig. 5). While the acini were usually unaffected by the procedure, a slight increase in luminal size and widened intercellular canaliculi were observed at 20 h after infusion.

A mild inflammatory cell-infiltrate, primarily consisting of polymorphonuclear leukocytes and macrophages, was present in the connective-tissue stroma 20 h after ductal filling. At 30 h, the number of these cells was reduced and the appearance of the glandular tissue was generally the same as that of the control. In a few areas, however, the luminal dilation of ducts and acini persisted, and numerous inflammatory cells were still present in the connective tissue. The number of mast cells and plasma cells appeared increased at later times, in addition, occasional eosinophils were seen.

In glands subjected to heavy parenchymal filling, both the irregularity of the luminal border and the dilation of the intralobular ducts, although reduced, were still present at 30 h after infusion. The changes in the ducts were similar to those observed after ductal filling (cf. Figs. 4, 5), but occurred more

frequently and were more pronounced following heavy parenchymal filling.

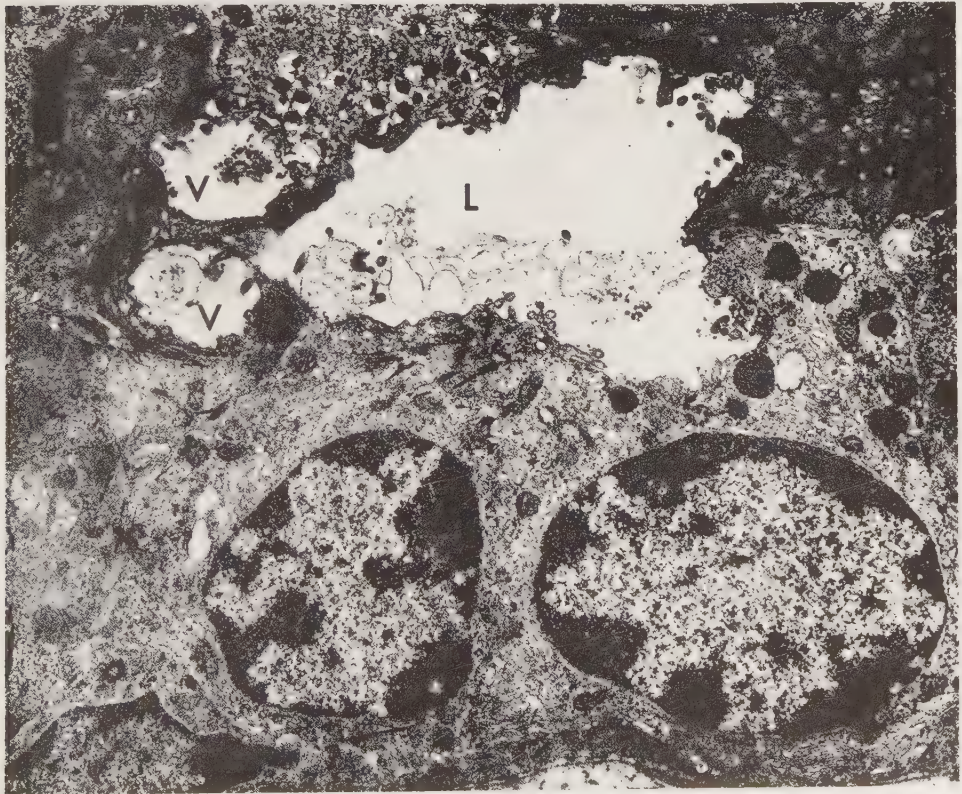
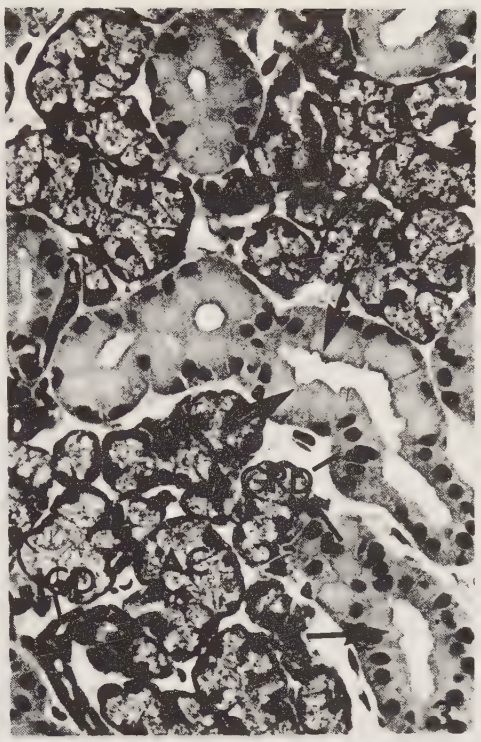
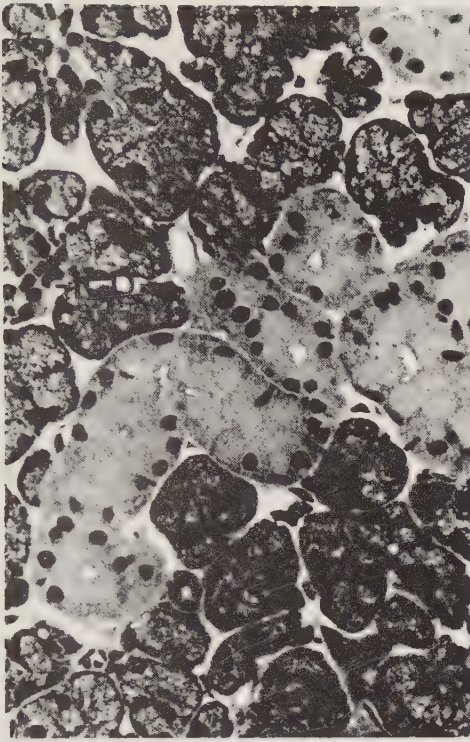
Heavy parenchymal filling frequently resulted in alterations in the acini. Irregular masses of fused secretory granules were seen in the supranuclear cytoplasm throughout the recovery period (Fig. 6), although they were particularly prominent at early times (10 and 20 h). Eventually, larger spherical vacuoles filled with apparent secretory product were present in some acinar cells. Occasionally, these vacuoles were seen in continuity with the luminal space. Dilated lumina and widened intercellular canaliculi were frequently observed at 20 h after infusion. Release of secretory granules at the lateral cell surface occurred occasionally (Fig. 7), and in some cases, the intercellular spaces between acinar cells appeared widened.

An inflammatory cell-infiltrate was already present in the overfilled glands at 10 h after infusion (Fig. 8). Initially, it consisted of polymorphonuclear leukocytes and macrophages, which frequently contained large vacuoles with a variety of cellular debris including apparent acinar secretory product. The infiltration was heavier than that observed after ductal filling and reached a peak at about 20 h, after which a successive decrease in the number of inflammatory cells was noted. Mast cells were prominent at later times, primarily located in the connective

Fig. 2. Light micrograph of control gland showing normal appearance of parenchymal tissue. Granular ducts (GRD), intercalated ducts (ICD), acini (AC). H&E, 2.5 μ m section, $\times 360$.

Fig. 3. Light micrograph of submandibular gland 10 h after heavy parenchymal filling with water-soluble radiographic contrast medium. The acini (AC) appear normal. Dilation of both intercalated ducts (ICD) and granular ducts (GRD) is seen. The luminal border of the granular ducts shows a scalloped appearance (arrows). H&E, 2.5 μ m section, $\times 360$.

Fig. 4. The luminal border of the intercalated ducts is irregularly enlarged, resulting in the appearance of vacuoles (V) in the apical cytoplasm of the duct cells. The desmosomes (D) have a normal appearance. Lumen (L). Electron micrograph, 20 h after ductal and slight parenchymal filling, $\times 10,000$.



tissue surrounding the larger vessels and ducts. An increase in the number of plasma cells was seen, and some infiltration of eosinophils occurred.

In some areas of the overfilled glands, the size of the acinar and intralobular duct cells was reduced. This was rarely seen at early times, but was quite frequent at seven days after infusion, when some atrophy of the glandular components was apparent (Fig. 9). The acinar cells appeared compressed and their content of secretory granules was reduced (Fig. 9). Many of the granular duct cells had a decreased number of dense granules located in their apical cytoplasm (Fig. 10). At this time, a proportional increase in the connective-tissue components was noted in the altered parts of the gland, and macrophages were frequently present in these areas. In addition, there was an apparent increase in the number of small blood vessels (Fig. 9).

In one gland, the connective-tissue stroma was particularly prominent and marked atrophy of the glandular components occurred. In many areas, no acini could be recognized; in others, the acinar cells were partially or totally depleted of their secretory granules. Dilatation of the intralobular ducts was extensive and inflammatory cells were often present in the luminal space (Fig. 11). Compression of the granular duct cells was apparent and depletion of their secretory granules occurred frequently.

At 10 days after infusion, the overfilled glands generally had a normal appearance, although a few areas with parenchymal atrophy and connective-tissue proliferation were still present. No inflammatory infiltrate was observed, however.

The interlobular ducts were usually unaffected although occasional ducts from overfilled glands appeared slightly dilated at early times after infusion. The epithelium of the main excretory duct appeared normal at all times during the recovery period. A moderate inflammatory infiltrate, most prominent in the overfilled glands, was present in the surrounding connective tissue at 20 h after infusion.

The appearance of the contralateral control glands was at all times similar to that described by other investigators (Scott & Pease 1959, Tamarin & Sreebny 1965). Furthermore, no morphological alterations were seen at 10 h, 30 h or 4 days in glands which had been cannulated but not subjected to infusion with radiographic contrast medium (Omnell & Qwarnstrom 1983).

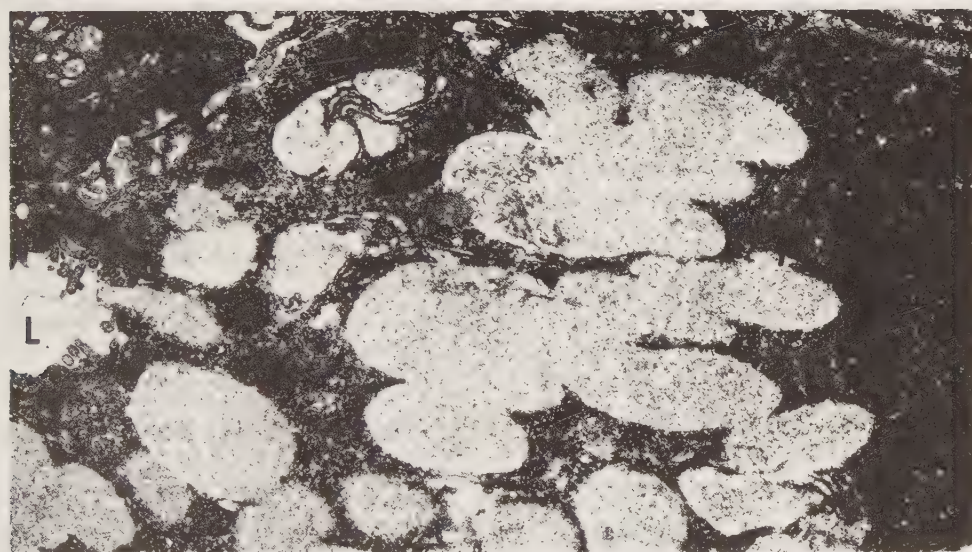
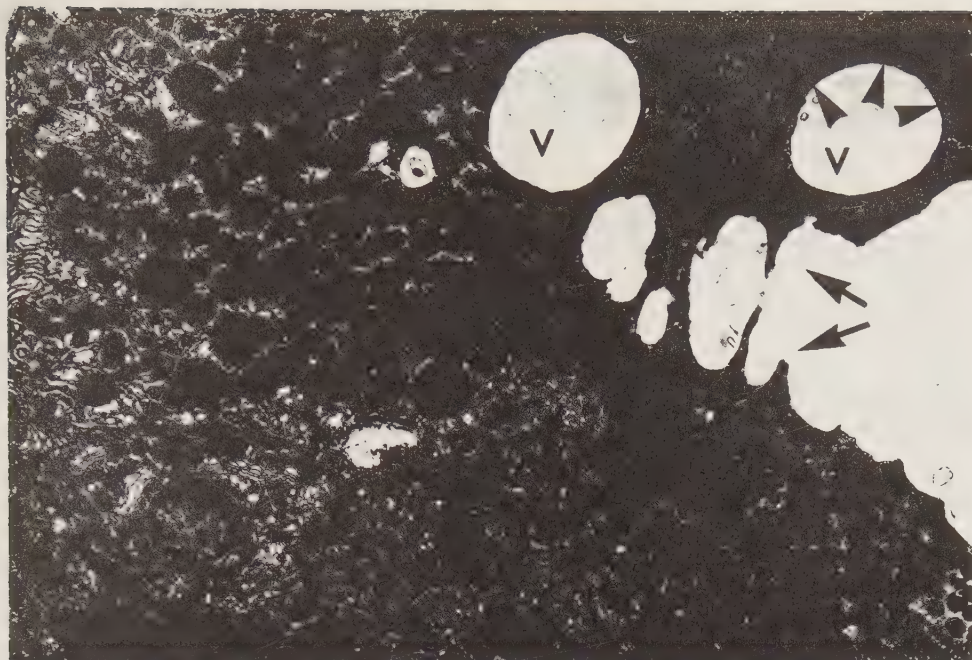
Discussion

After ductal filling, the recovery of the glandular tissue was rapid and consisted mainly of a successive decrease of the luminal diameter. At 20 h and 30 h hours after infusion, a mild

Fig. 5. Pronounced scalloping of the luminal border of the granular ducts (arrows) results in compression of the secretory granules (arrowheads) and the presence of apparent vacuoles (V) in the apical cytoplasm. Electron micrograph, 30 h after ductal and slight parenchymal filling, $\times 5,000$.

Fig. 6. Confluence of secretory granules (SG) in the supranuclear cytoplasm of acinar cells was seen throughout the recovery period. Nucleus (N), lumen (L). Electron micrograph, 4 days after heavy parenchymal filling, $\times 15,400$.

Fig. 7. Discharge of secretory granules (arrows) into the intercellular spaces (ICS) between acinar cells (AC) occurs occasionally. Lumen (L). Electron micrograph, 20 h after heavy parenchymal filling, $\times 14,200$.



28°

inflammatory reaction was seen, after which the tissue generally had a normal appearance. The recovery of the glands subjected to heavy parenchymal filling was much slower and the inflammatory reaction was considerably stronger. In addition, degenerative changes in the parenchyma and apparent connective-tissue proliferation were seen at later times in the overfilled glands. The variable changes observed in different areas of the same gland probably coincide with the uneven distribution of the contrast medium during infusion and the noted variations in the initial damage (Qwarnstrom & Hand 1982).

The luminal dilation of the intralobular ducts was reduced at early post-infusion times. Some decrease in the compression of the duct cells probably occurs as soon as the luminal pressure is reduced. An immediate recovery of the distension of the luminal system in dog submandibular gland has been noted when lowering the outflow level after induced hydrostatic pressure (Emmelin et al. 1977). The intercellular junctions also apparently respond to reduction in pressure. Desmosomes, frequently widened immediately after heavy parenchymal filling of the gland (Qwarnstrom & Hand 1982), had a normal

appearance in both groups of animals at 10 h. Functionally, the junctional complexes appear to be largely intact following release of the pressure, since no serum albumin could be detected in stimulated saliva collected immediately after either degree of filling (Qwarnstrom et al. in preparation). During infusion, however, contrast medium containing a macromolecular tracer (horseradish peroxidase) penetrated the tight junctions and reached the intercellular spaces (Qwarnstrom & Hand 1982).

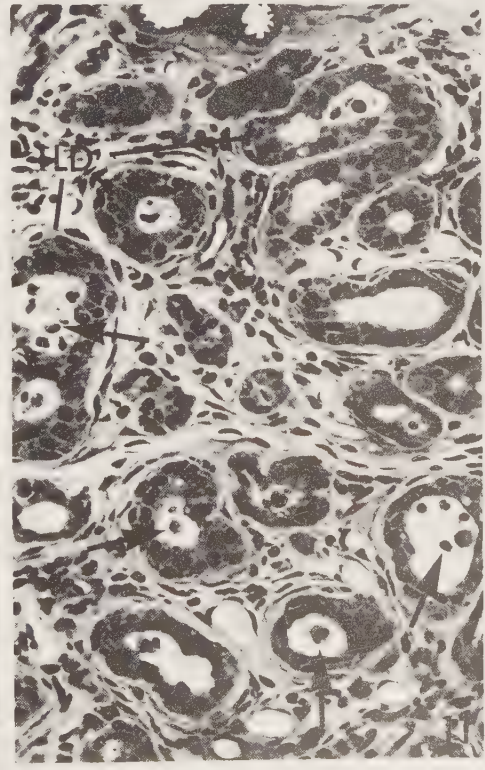
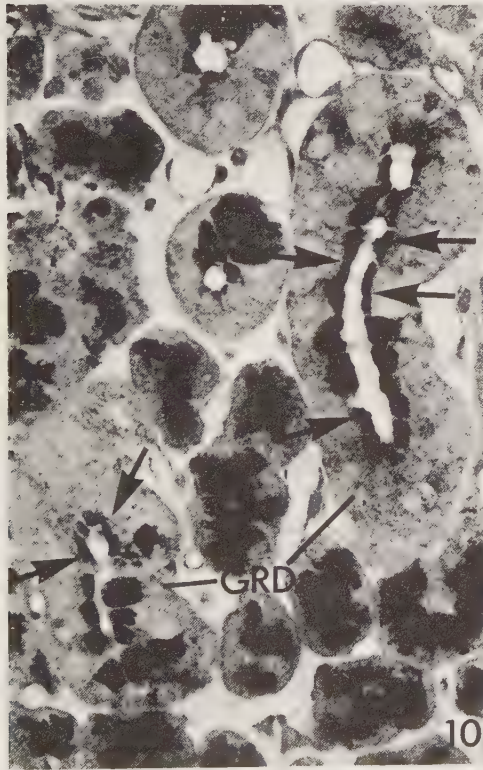
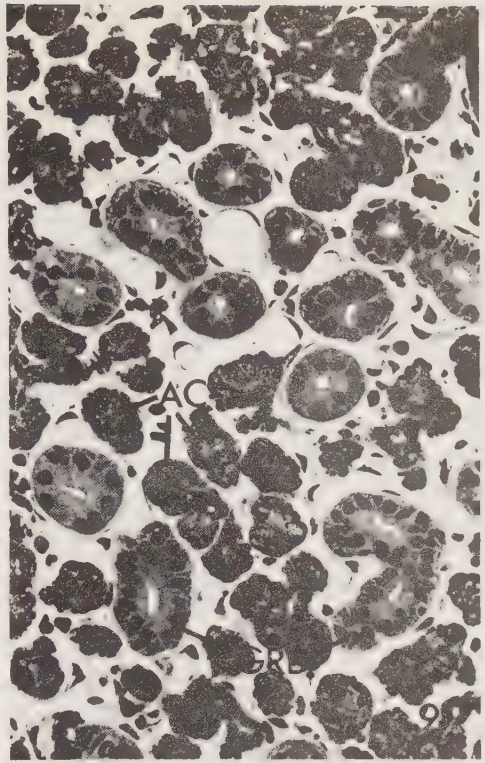
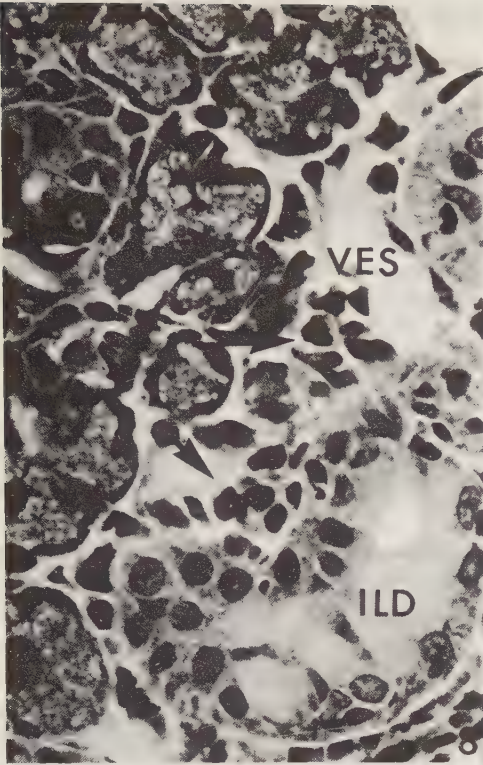
Persistence of the ductal dilation and the irregularity of the luminal border could reflect damage to the cellular membrane as a result of the induced intraluminal pressure. The vacuoles observed in the apical part of the duct cells probably constituted extensions of the lumen, as they frequently contained material similar to that observed in the luminal space, and microvilli were present on the limiting membrane. In the granular ducts, these changes may also be influenced by alterations in the secretory pattern. Fusion and release of the secretory granules caused by mechanical stress, resulting in the addition of membrane to the apical cell surface, could account for the persistence of the scalloped ap-

Fig. 8. A large number of inflammatory cells (arrows) are present, particularly next to intralobular ducts (ILD) and blood vessels (VES). Light micrograph, 20 h after heavy parenchymal filling. Giemsa stain, 2.5 μ m section, $\times 700$.

Fig. 9. An increase in the connective tissue and the number of blood vessels is apparent in some areas at later times. In addition, a reduction in size of the parenchymal cells is noted. Acini (AC), granular ducts (GRD). Light micrograph, 7 days after heavy parenchymal filling. H&E, 2.5 μ m section, $\times 290$.

Fig. 10. The granular duct cells (GRD) are reduced in size and fewer granules are present in their apical cytoplasm (arrows). Light micrograph, 4 days after heavy parenchymal filling. Periodic acid-Schiff stain, 2.5 μ m section, $\times 490$.

Fig. 11. Considerable atrophy of the parenchymal cells occurs in some areas. Dilation of the intralobular ducts (ILD) is considerable and inflammatory cells (arrows) are present in the lumina. Acini are difficult to identify. Light micrograph, 7 days after heavy parenchymal filling. H&E, 2.5 μ m section, $\times 290$.



pearance in these ducts. The decrease in the number of large, mature granules* in the granular duct cells could also be explained by this phenomenon. A loss of granules in the granular ducts has been observed after duct ligation (Tamarin 1967).

The confluence of secretory granules and vacuole formation occurring in the acinar cells were probably not directly caused by the intraglandular pressure since such changes were not observed immediately after infusion (Qwarnstrom & Hand 1982). It is more likely that they were induced secondarily, as a result of alterations in the secretory mechanism. Similar changes have been seen in salivary glands after starvation (Hand 1972), and in streptozotocin-induced diabetes (Cutler et al. 1979). These phenomena also occur after repeated administration of autonomic antagonists (Donath et al. 1974) and thus, could be due to impaired release of secretory granules. It is possible that changes in the apical membrane, induced by the applied luminal pressure, can have a similar effect. Formation of vacuoles in acinar cells after secretagogue administration subsequent to degranulation (Garrett et al. 1978) were attributed to induced alterations in membrane permeability.

The observed inflammatory reaction was probably a result of both cellular damage and alterations in the secretory pattern, causing accumulation of cellular debris and secretory product in the interstitium. The response was quite similar to that seen after duct ligation (Bhaskar et al. 1966, Harrison & Garrett 1976, Tamarin 1979), with an early infiltrate of macrophages and polymorphonuclear leukocytes and a later increase in plasma cells

and eosinophils. The numerous mast cells observed at later times may partly be responsible for the apparently enhanced angiogenesis (Azizkhan et al. 1980, Taylor & Folkman 1982). The presence of inflammatory cells in the ductal lumina, previously seen after duct ligation (Tamarin 1979), was suggested to be due to a chemotactic effect of the retained secretory product. Stagnation of secretory material in the ducts of glands severely altered by infusion could induce the similar phenomenon observed in this study.

Some of the observed alterations could be due to, or affected by, the presence of the contrast medium. It is known that radiographic contrast medium induces damage when injected intravascularly (Mersereau & Robertson 1961). Although diatrizoate, the component present in Renografin, has been found to have relatively low toxicity (Hoppe et al. 1956, Hoppe 1959, Hoppe & Archer 1960), the medium causes an increased permeability of the endothelial wall which can result in perivascular edema (Harrington & Wiedman 1965). Damage to the ductal epithelium observed after retrograde filling of the pancreas was, to a great extent, attributed to the presence of the infused water-soluble contrast medium (Stolte et al. 1981). Similar changes occurring in the salivary gland parenchyma following infusion could contribute to the observed morphological alterations and the inflammatory infiltration. The length of time the tissue is exposed (Olsson 1954, McConnell & Mersereau 1964, Stolte et al. 1981), as well as the amount of contrast medium administered (Morino et al. 1959), are of importance for the severity of the induced changes. In the overfilled glands, the opaque dye will have penetrated the intercellular junctions to reach the interstitial tissue (Qwarnstrom & Hand 1982), which could account for some of the variable reactions noted between glands subjected to different degrees of filling. Due to the low toxicity and rapid

* Radioautographic studies of L-³H-fucose incorporated into glycoproteins of mouse submandibular gland granular tubule cells by T. G. Lima and A. Haddad (*Cell Tiss. Res.* **220**, 405-425, 1981) demonstrated that the larger electron dense granules are more mature than the smaller granules.

diffusion of the contrast medium, the alterations induced by its presence in the tissue are probably of minor significance. It is possible, however, that in combination with an induced mechanical stress, the effect may be greater (Scatliff et al. 1963).

Heavy parenchymal filling with the water-soluble contrast medium eventually resulted in parenchymal atrophy and an apparent increase in the connective-tissue stroma. A decrease in function of the glandular tissue, such as occurs following liquid diet (Hall & Schneyer 1964, Sreebny & Johnson 1968, Johnson & Sreebny 1971; Hand & Ho 1981), is known to induce atrophy of the parenchyma (Wells 1967). Both atrophy (Sodicoff et al. 1974) and replacement of the parenchyma by connective tissue (Nicolatou 1981) occur in salivary glands after x-irradiation, as well as in streptozotocin-induced diabetes (Cutler et al. 1979). Various degrees of parenchymal atrophy and connective-tissue proliferation can also be induced by duct ligation, both in salivary glands (Tamarin 1971, Harrison & Garrett 1972, 1976, Donath et al. 1973), and in the exocrine pancreas (Churg & Richter 1971). These changes, primarily seen at later times after infusion, could also be due, in part, to secondary alterations occurring during the recovery period (Bhaskar et al. 1966).

The observed changes are, most likely, primarily caused by the increased intraglandular pressure. The severity of the changes and the ability of the gland to recover are probably determined to a great extent by the initial damage and the following inflammatory reaction, and to a lesser degree by the presence of the contrast medium. Regeneration and reorganization of the parenchyma occurred within 7 days in most areas, even after heavy parenchymal filling, and the function of the gland had generally returned to normal at this time (Qwarnstrom et al., in preparation). Our results indicate that ductal and slight parenchymal filling with water-soluble radiographic

contrast medium causes relatively mild changes, from which the glandular tissue promptly recovers. Heavy parenchymal filling, however, induces pronounced changes in the tissue which may result in longer lasting alterations.

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A morphologic study of the recovery of the rat submandibular gland after retrograde infusion

II. Lipid-soluble radiographic contrast medium

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Lipid-soluble radiographic contrast medium was infused retrogradely through the main excretory duct of the rat submandibular gland until ductal and slight parenchymal filling or heavy parenchymal filling was achieved, as determined by the developing intraglandular pressure. The glandular tissue was fixed by vascular perfusion at different times following infusion, and examined by light and electron microscopy. Dilation of the intralobular ducts and scalloping of the luminal border were pronounced after both degrees of filling. Widening of the acinar lumina and intercellular canaliculi occurred early. Masses of fused secretory granules were commonly seen in the cytoplasm of the acinar cells and formation of vacuoles occurred frequently. In some acinar cells, densely packed granules filled the major part of the cytoplasm. Large lysosomes and autophagic vacuoles, frequently present in the various parenchymal cells, often contained apparent contrast medium. In addition, widening of the intercellular spaces between parenchymal cells was seen, particularly in the acini and intercalated ducts. An inflammatory cell-infiltrate, primarily comprised of polymorphonuclear leukocytes, was particularly prominent at 20 h and 30 h after infusion. The changes were generally more pronounced and persisted for a longer time in glands subject to heavy parenchymal filling. Large cyst-like spaces surrounded by inflammatory cells were seen in the lobules and in the interlobular connective tissue of these glands. At later times, areas containing apparent contrast medium surrounded by macrophages, were frequently observed in the connective-tissue stroma. Atrophy of the parenchymal cells was seen later, after heavy parenchymal filling, and a proliferation of the connective tissue had occurred. The changes were most probably caused by the elevated intraglandular pressure induced during infusion, and the subsequent cell damage and inflammatory reaction. A foreign body reaction, induced by the retained lipid-soluble contrast medium, was probably partly responsible for the morphological alterations observed, following infusion.

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In clinical sialography, lipid-soluble radiographic contrast medium is used for retrograde filling of the salivary glands to visualize the ductal system and the parenchyma (Osmer & Pleasants 1966, Trester 1968, Sazama 1973, Blair 1976). It differs considerably in physical properties from the water-soluble medium. In addition to the difference in solubility, the viscosity of the lipid-soluble contrast medium is about six times that of the water-based compound.

It was found that the development of the intraglandular pressure during infusion of this medium into the rat submandibular gland was reproducible and characteristic, and was different from that measured during administra-

tion of the water-soluble contrast medium (Qwarnstrom et al. 1981). As with the water-soluble medium, it was possible to correlate different degrees of filling of the gland, as seen in the sialogram, to different phases of the pressure curve. It was also demonstrated that the immediate effect of the sialographic procedure on the glandular tissue was much more severe when the lipid-based compound was used (Qwarnstrom & Hand 1982b).

This investigation studied the recovery of the infused gland, and examined the long-term effects of the sialographic procedure, using the lipid-soluble radiographic contrast medium.

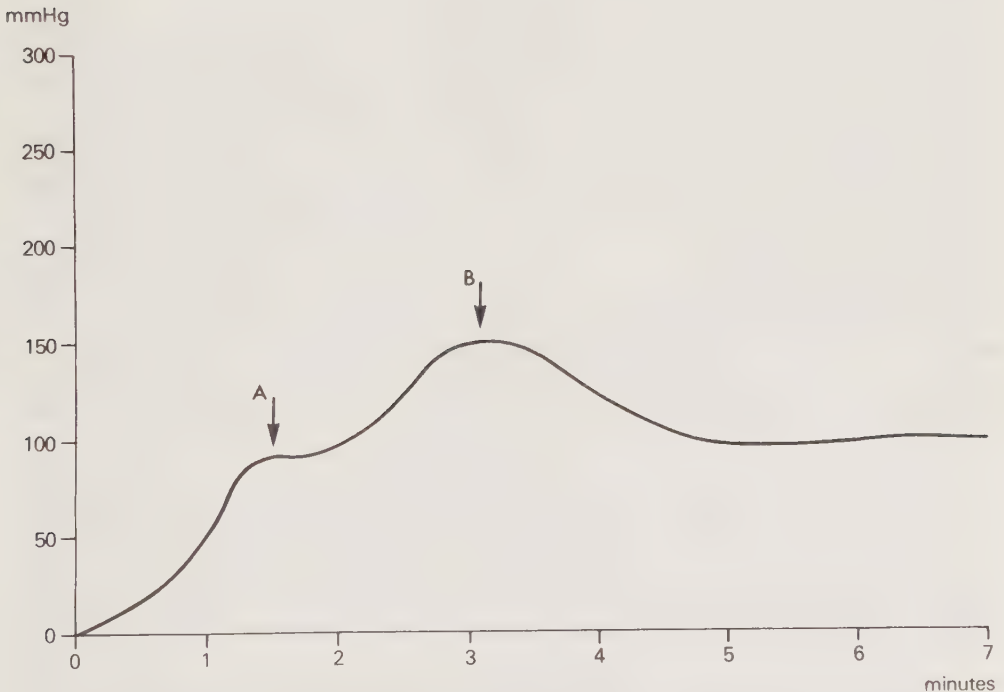


Fig. 1. Typical intraglandular pressure development during retrograde infusion of lipid-soluble radiographic contrast medium. The arrows indicate where infusion was stopped to achieve ductal and slight parenchymal filling (A) and heavy parenchymal filling (B), respectively.

Material and methods

A total of 27 male Sprague-Dawley rats, weighing between 225 and 500 g, were used. General anesthesia was obtained by intraperitoneal injection of sodium pentobarbital (45 mg/kg b.w.). Intraoral cannulation of the main excretory duct of the left submandibular gland was done according to Omnell and Qwarnstrom (1983).

The intraglandular pressure was measured during continuous retrograde infusion of Lipiodol UF (Guerbet; iodine content 480 mg/ml; viscosity 25 mPa·s at 36°C) at a rate of 0.008 ml/min. The system was the same as that used previously (Qwarnstrom & Hand 1982b, see Fig. 1 in Qwarnstrom & Hand 1982a). The pressure recording device consisted of a displacement transducer (Bentley Trantec, Model 800 No. 10118) which was connected to an amplifier (Sandborn, Model 350-1100B) and a recorder (LKB 2210). The syringe fitted to the infusion pump (Sage Instruments, syringe pump Model 341A) and the tubing connecting it to the gland were filled with the contrast medium. Other parts of the system were filled with distilled water. Calibration was done as described previously (Qwarnstrom et al. 1983).

In 11 animals, infusion was continued until a ductal and slight parenchymal filling was achieved, according to the development of

the intraglandular pressure (Qwarnstrom et al. 1981). In 16 animals, the gland was purposely overfilled (heavy parenchymal filling) (Fig. 1).

For 2 days following the procedure, the rats were given ground chow, after which they were fed their normal pelleted diet. Animals subjected to the different degrees of filling were killed at various times after infusion of the contrast medium (10, 20, 30 h and 4, 7, 10 days). The submandibular glands were fixed by vascular perfusion and the tissue was prepared for light and electron microscopic examination as previously described (Qwarnstrom et al. 1983). The right (uninfused) submandibular gland from each animal served as a control.

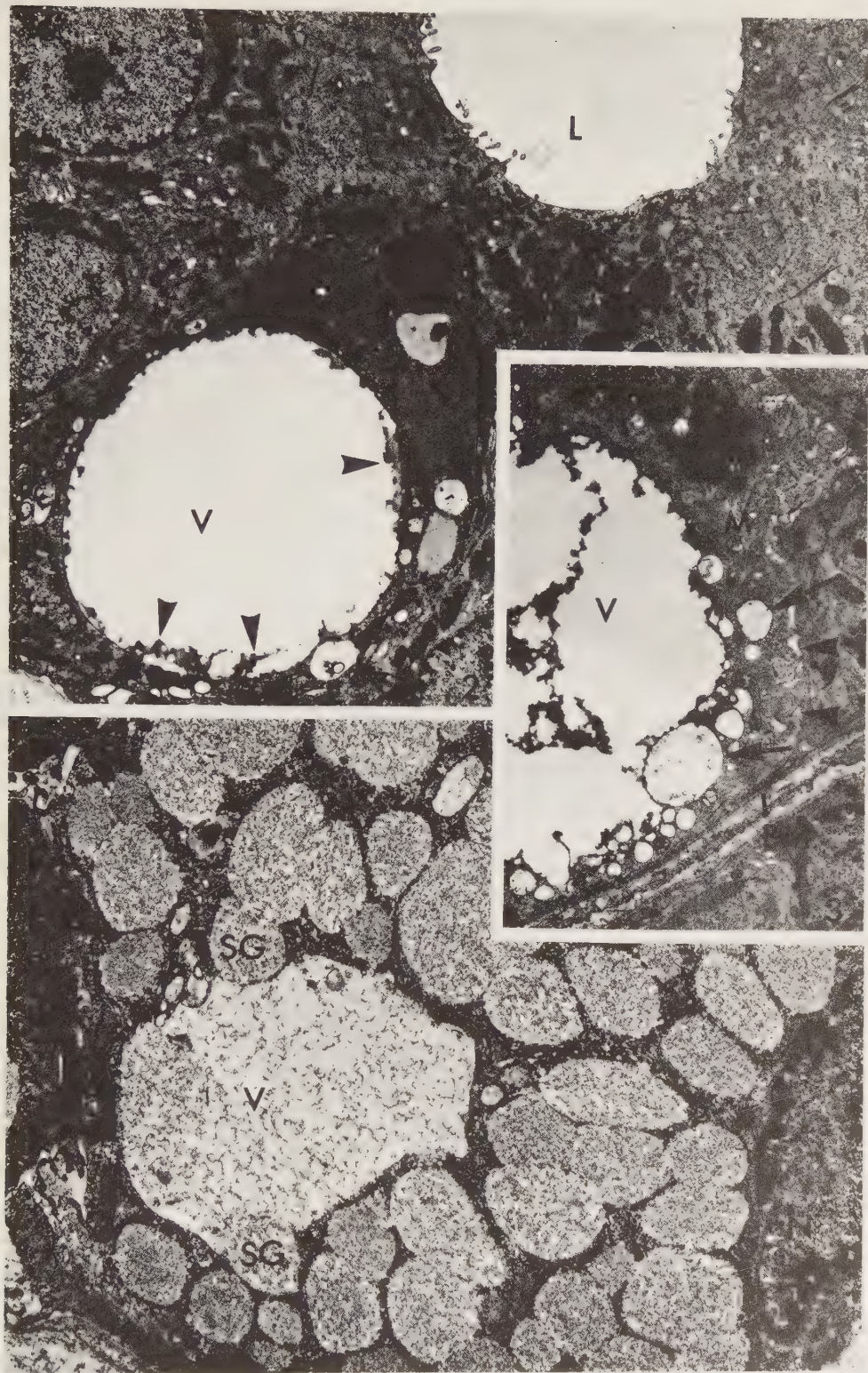
Results

Dilation of the intralobular ducts was clearly seen at 10 h and 20 h after infusion of lipid-soluble radiographic contrast medium. As shown for the water-soluble contrast medium (Qwarnstrom et al. 1983), these changes were most prominent in the overfilled glands. Pronounced scalloping of the luminal border of the granular ducts frequently resulted in the appearance of empty vacuoles in the apical cytoplasm. The intercalated ducts showed considerable dilation, particularly in the pro-

Fig. 2. Striated duct with several small (arrows) and one large vacuole (V). Electron-dense material is present in the periphery of the large vacuole (arrowheads). The lumen (L) has a normal appearance. Electron micrograph, 30 h after heavy parenchymal filling. $\times 5,600$.

Fig. 3. A large vacuole (V) containing electron-dense material is present in a macrophage (M) located in the intercellular spaces of a striated duct (arrow heads). Numerous small vacuoles (arrows) are also present in the macrophage. Basement membrane (BM). Electron micrograph, 20 h after heavy parenchymal filling. $\times 5,600$.

Fig. 4. Secretory granules (SG) appear to empty into a large vacuole (V) present in the basal cytoplasm of an acinar cell. Intercellular space (ICS), nucleus (N). Electron micrograph, 30 h after heavy parenchymal filling. $\times 10,300$.



ximal part of the duct, and apparent vacuoles were also present in the apical cytoplasm of these cells.

Basally located vacuoles were present in the intralobular ducts, particularly in the striated ducts (Fig. 2). They were most commonly seen in the overfilled glands, but also occurred after ductal filling. The vacuoles usually were localized in the cytoplasm of the duct cells, but, occasionally, were contained within macrophages present in the intercellular spaces of these ducts (Fig. 3). Electron-dense material with a smooth or somewhat granular appearance was frequently present in these vacuoles, most commonly located in the periphery.

Dilation of the acinar lumina and widening of the intercellular canaliculi were occasionally seen. These changes were most prominent in areas near the acinar-intercalated duct junction, and were observed at 10 h in the overfilled glands, and in both groups of animals at 20 h after infusion. An increase in the release of secretory granules at the apical cell surface occurred. More common, however, was confluence of mucous-secretory granules in the acinar cells, frequently seen at early times (10, 20 h). In the overfilled glands, this often led to the formation of cytoplasmic vacuoles in the acinar cells (Fig. 4). At later times an increase in the number of acinar secretory granules was frequently observed, resulting in densely packed granules that in some cells filled the major part of the cytoplasm (Fig. 5).

Large lysosomes and autophagic vacuoles containing degenerating cellular organelles

and electron-dense material were quite commonly seen in the parenchymal cells. They were particularly prominent in the acinar cells (Fig. 6) and occurred most frequently in the overfilled glands.

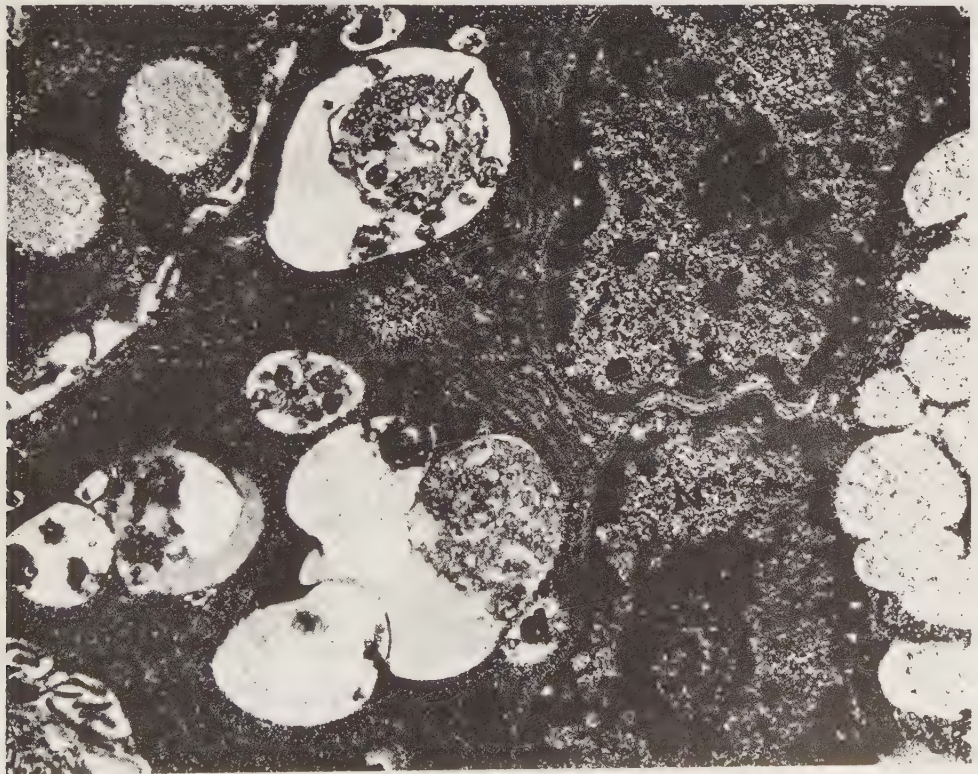
Widening of the intercellular spaces was commonly seen at early times (10, 20 h) in the acini (Fig. 7) and the intercalated ducts. In addition, displacement and extensive folding of the basement membrane occurred in these segments. This was particularly common in the most damaged areas, where it was also pronounced at later times.

An inflammatory cell-infiltrate, mainly consisting of polymorphonuclear leukocytes and macrophages containing vacuoles with a content of variable density, was present at 10 h after infusion. The infiltrate reached a peak at 20 h after ductal filling when mast cells, plasma cells and numerous eosinophils were also present in the connective tissue. At 30 h, the infiltrate was markedly decreased and the morphological changes in the parenchyma were less pronounced. Only occasional inflammatory cells were seen in these glands at 4 days and the parenchymal tissue generally had a normal appearance at this time.

In glands subjected to heavy parenchymal filling, the infiltration of inflammatory cells was much more pronounced and the inflammatory response lasted longer. Cyst-like spaces surrounded by a large number of inflammatory cells were seen in the interlobular connective tissue and within the lobules of these glands (Fig. 8). Macrophages and polymorphonuclear leukocytes were most frequently present; in addition, numerous

Fig. 5. Densely packed granules are seen in some acinar cells at later times. Intercalated duct (ICD). Electron micrograph, 30 h after heavy parenchymal filling, $\times 4,450$.

Fig. 6. Acinar cells containing autophagic vacuoles in the cytoplasm (arrows). Intercellular spaces (ICS), nuclei (N). Electron micrograph, 20 h after ductal and slight parenchymal filling, $\times 12,600$.



eosinophils were observed. Plasma cells were located in the periphery of these cyst-like structures. Mast cells were also occasionally present. More commonly, however, these cells were seen adjacent to the larger ducts and vessels.

At later times the inflammatory cells were seen in the lobules between the parenchymal components. Quite often they were present inside the basement membrane, between the different cell types (Fig. 9). Macrophages were particularly numerous, frequently containing phagocytic vacuoles with acinar secretory material or apparent contrast medium. A large number of macrophages was also seen in the connective tissue, where several of these cells frequently enclosed rounded spaces of different sizes (Figs. 10, 11). The macrophages totally circumscribed these spaces, whereas other types of inflammatory cells were present in the surrounding connective tissue. Scattered electron-dense material with a granular appearance was present in some areas of the enclosed spaces.

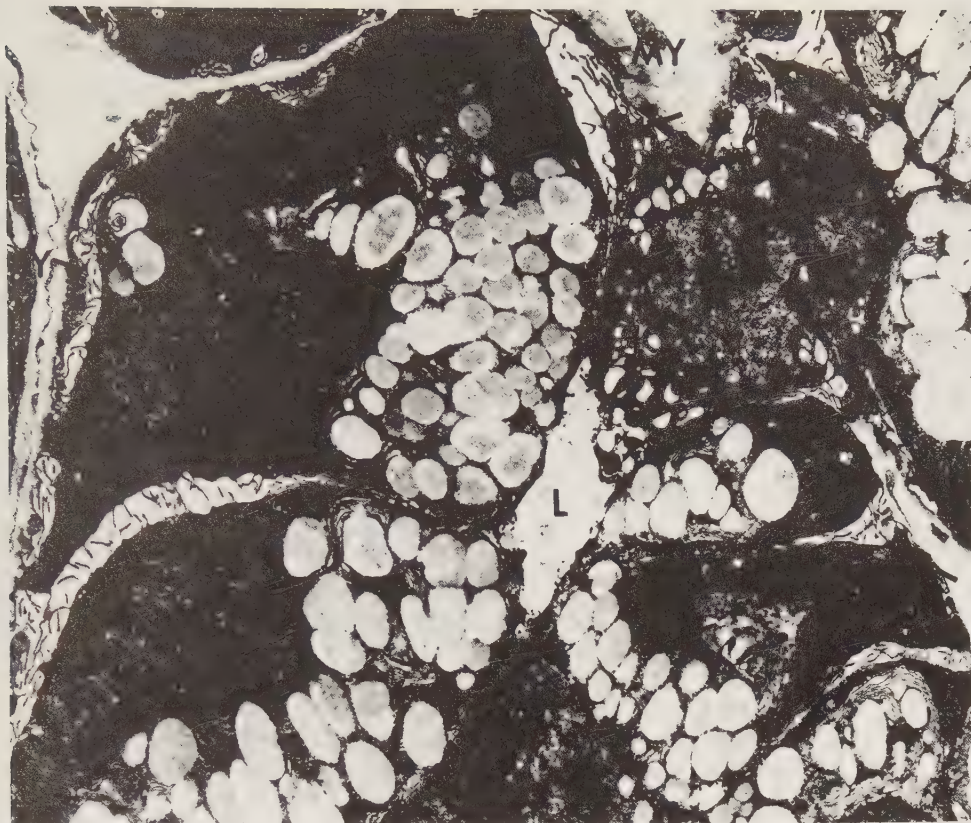
An increase in the connective tissue of the overfilled glands was observed initially at 20 h after infusion and became successively more pronounced. Eventually, a decrease in size of the parenchymal cells was seen in some areas. This was most frequent in the acini where the content of secretory granules was depleted. Vacuoles of different sizes, often containing peripherally located electron-dense material, were present in the acinar cells (Fig. 11). Autophagic vacuoles were occasionally seen,

predominantly in the acini and intercalated ducts. In some areas these vacuoles were large and caused deformation of the parenchymal cells. The granular duct cells appeared compressed and reduced in size and the number of large granules in their cytoplasm was decreased, as shown previously (Qwarnstrom et al. 1983). In addition, the cells at the acinar-intercalated duct junction containing heterogeneous granules (Qwarnstrom & Hand 1983) were increased in number. This was occasionally seen at 20 h and became more pronounced at later times, especially in areas where the parenchyma had a more normal appearance (Fig. 12).

Variations in the severity of the changes were striking. At 4 days, the acini and the intralobular ducts in some areas had a normal appearance, whereas in others, the parenchyma showed considerable atrophy. These changes were still present at 7 days in some of the overfilled glands. Rounded spaces surrounded by macrophages were frequently seen in a prominent connective-tissue stroma (Fig. 13). Occasionally, the macrophages enclosed groups of deformed cells. In some areas, the alterations were so severe that distinction between the parenchymal cell types was impossible to make (Fig. 14). Identifying features, such as basal infoldings in the duct segments and secretory granules in the acinar and granular duct cells, were not observed. The atrophic parenchymal cells frequently contained vacuoles in their cytoplasm (Fig. 14). In addition, the intercellular spaces be-

Fig. 7. Dilation of the intercellular spaces (arrows) between acinar cells is apparent. Lumen (L), myoepithelial cell (MY), granular intercalated duct cell (GIC). Electron micrograph, 30 h after ductal and slight parenchymal filling, $\times 4,500$.

Fig. 8. Cyst-like spaces, surrounded by numerous inflammatory cells, are present in the interlobular connective tissue (arrow) and in the glandular lobules (double arrow). A diffuse inflammatory infiltrate (arrowheads) is seen. Acini (AC), intercalated duct (ICD). Light micrograph, 20 h after heavy parenchymal filling, $2.5 \mu\text{m}$, Giemsa stain, $\times 380$.



tween these cells were widened and frequent displacement and pronounced folding of the basement membrane were seen.

In animals killed 10 days after heavy parenchymal filling, the glandular tissue generally had a normal appearance, the same as that of the uninfused control glands. In some areas, however, atrophy of the parenchymal cells and an increase in the connective-tissue stroma were observed. In addition, cytoplasmic vacuoles were present in occasional acinar and intralobular duct cells.

The interlobular ducts were usually unaffected by the infusion of lipid-soluble radiographic contrast medium. However, at early times after overfilling, some of the smaller interlobular ducts were slightly dilated. The epithelium of the main excretory duct appeared normal at all times. An increase in the number of inflammatory cells in the surrounding connective tissue, particularly prominent at 20 h and 30 h after infusion, was noted after both degrees of filling.

Discussion

Either degree of filling with lipid-soluble radiographic contrast medium resulted in marked changes during the first week after infusion. Several of the observed alterations were the same as those present after infusion of water-soluble radiographic contrast medium, but were much more severe and

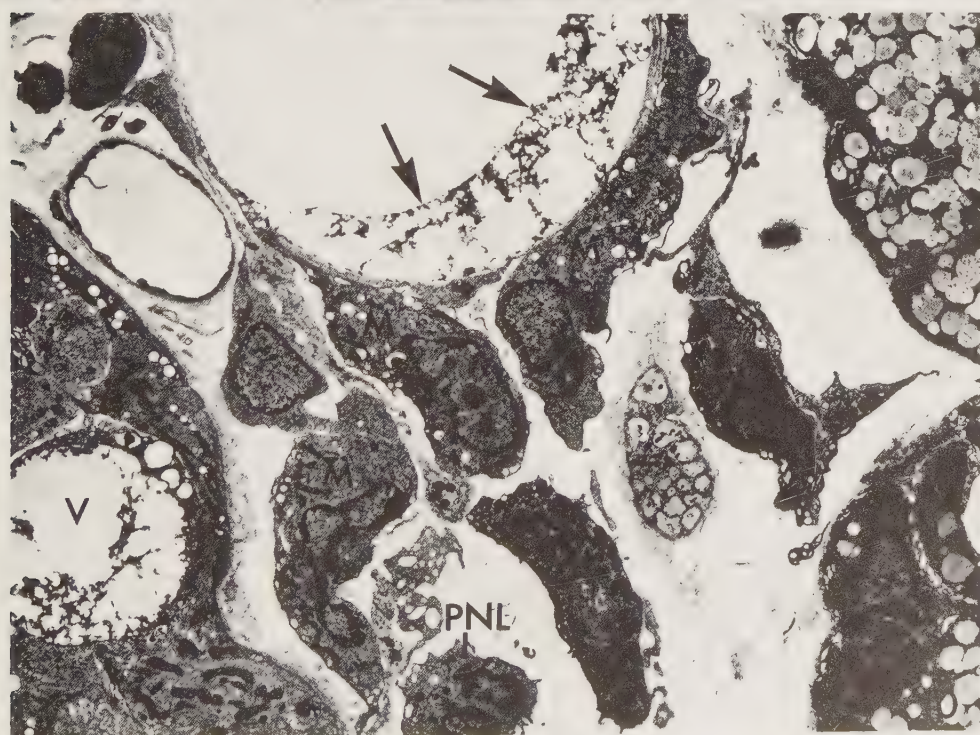
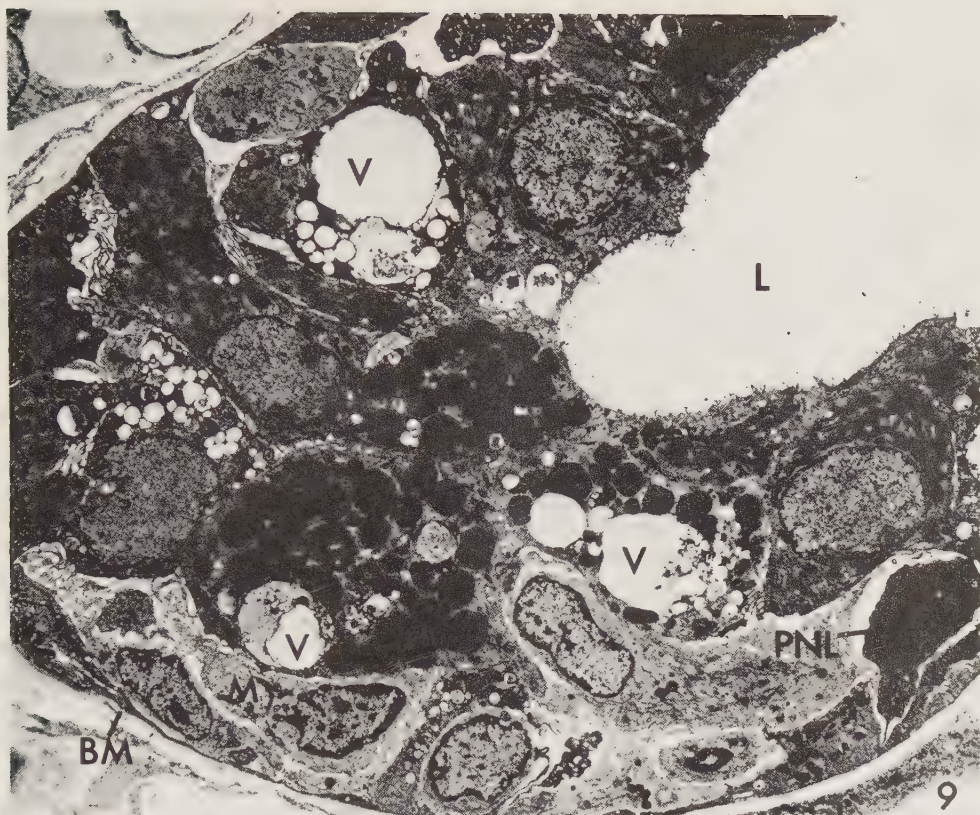
occurred more frequently. Thus, the dilation of the intralobular ducts was pronounced, and vacuoles in the apical cytoplasm of the duct cells were much more commonly seen. These vacuoles probably constituted extensions of the lumen formed as a direct result of the induced intraglandular pressure, since similar changes were seen immediately after infusion (Qwarnstrom & Hand 1982b).

The high intraluminal pressure probably has a severe effect on the secretory function, contributing to the variety of changes observed in the acinar cells. Widening of the intercellular spaces, quite frequently seen at early times, may be a result of changes in fluid and electrolyte transport. Changes in the configuration of the intercellular spaces in the gallbladder have been correlated with alterations in fluid and electrolyte transport (Kaye et al. 1966, Tormey & Diamond 1967). As acini and intercalated ducts are believed to be the main sites for water and electrolyte secretion in salivary glands (Young & Schneyer 1981), it is possible that the alterations observed after infusion could be due to such phenomena. In addition, release of secretory granules at the lateral cell surface, observed immediately after infusion (Qwarnstrom & Hand 1982b), could be partly responsible for the alterations in intercellular space morphology.

Confluence of secretory granules and subsequent vacuole formation were much more common following infusion of the lipid-

Fig. 9. Polymorphonuclear leukocytes (PNL) and macrophages (M) are present inside the basement membrane (BM) of a granular duct. The granular duct cells contain cytoplasmic vacuoles (V). The border of the lumen (L) has a scalloped appearance. Electron micrograph, 30 h after heavy parenchymal filling, $\times 2,100$.

Fig. 10. A rounded space in the connective tissue surrounded by macrophages (M) contains electron-dense material with a granular appearance (arrows). Polymorphonuclear leukocytes (PNL) and eosinophils (E) are also present in the connective tissue. Note vacuole (V) with electron-dense material in a striated duct cell. Acini (AC). Electron micrograph, 30 h after heavy parenchymal filling, $\times 2,950$.



soluble compound. This could probably be explained by more severe and more frequent changes in the apical membrane induced by the higher intraglandular pressure, thus leading to more pronounced alterations in the normal exocytotic process. The accumulation of large numbers of granules in some acinar cells after infusion could also be due to related changes in the apical membrane. This has previously been seen in the submandibular gland following sympathectomy (Garrett & Harrop 1976), suggesting that it can be due to an inability to release the secretory granules.

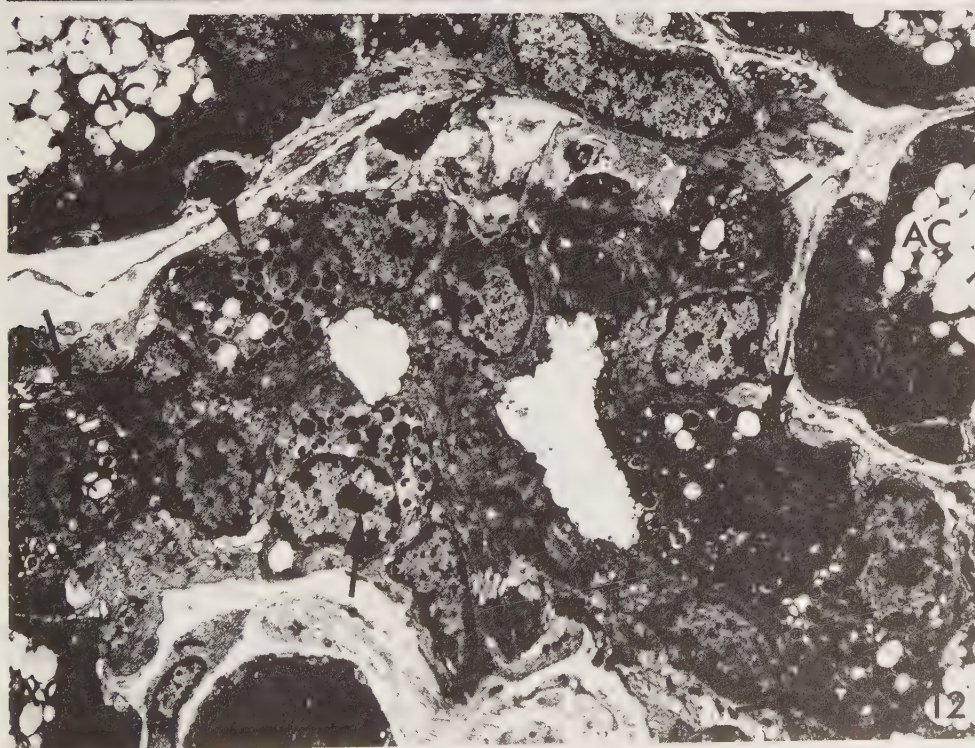
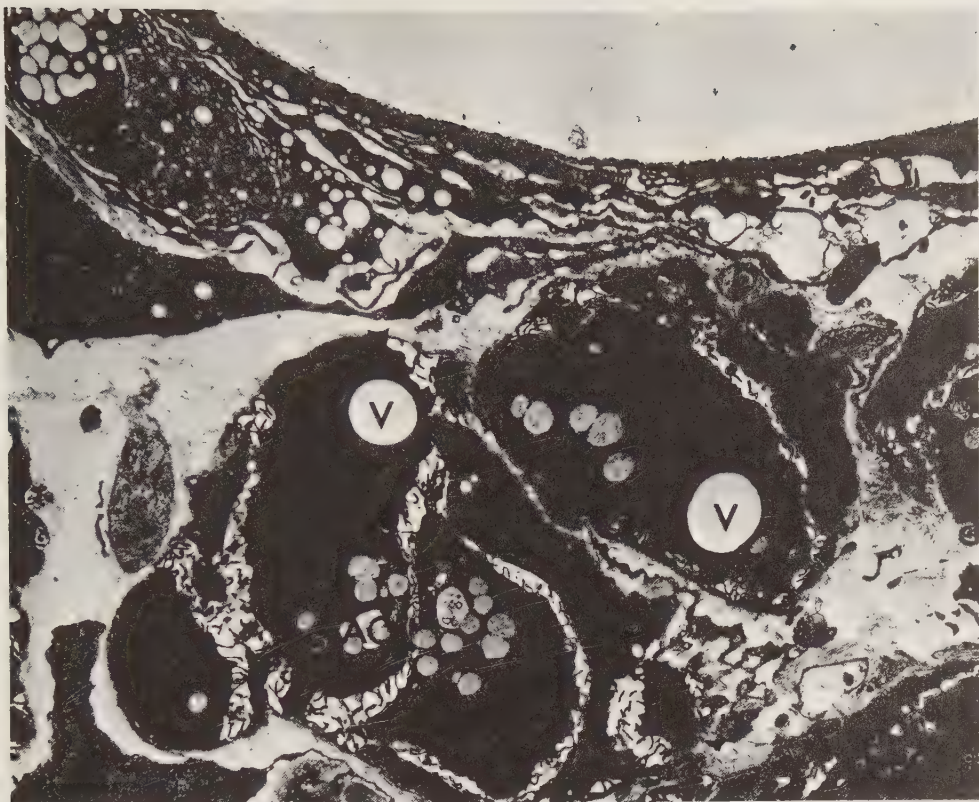
Formation of autophagic vacuoles in the parenchymal cells is probably a direct result of significant cellular damage caused by the infusion. Thus, it can be induced by x-irradiation (Pratt & Sodicoff 1972, Sholley et al. 1974, Sodicoff et al., 1974, Nicolatou 1981, Chomette et al. 1981), and occurs in the salivary glands (Tamarin 1967, Donath et al. 1973), and the exocrine pancreas (Churg & Richter 1971), following duct ligation. It may be partly the result of severe functional alterations, as it has been seen following administration of sympathetic antagonists (Donath 1973), and in a number of conditions known to alter the normal secretory pattern in exocrine glands, such as actinomycin D administration (Han 1967), acute starvation (Hand 1972), vinblastine treatment (Kelly et al. 1978), and liquid diet feeding (Hand & Ho 1981).

The pattern of the inflammatory reaction

was similar to that noted after infusion of the water-soluble medium although considerably more pronounced. The difference is probably mostly due to the more severe parenchymal changes induced by infusion of the lipid-based compound. The presence of inflammatory cells within the basement membrane has previously been observed in exocrine glands following x-irradiation (Sodicoff et al. 1974, Pratt & Sodicoff 1972), and in other experimental conditions known to induce cellular injury in salivary glands (Hand 1973, Schneyer & Wilborn 1976, Harrison & Garrett 1976, Tamarin 1979, Oliver et al. 1979, Hand & Ho 1981). It is likely, however, that the inflammatory response, to some extent, results from a local irritation caused by the presence of the contrast medium, as a severe inflammatory reaction is also seen after subcutaneous administration (Lilly et al. 1968). Retention of the lipid-based medium is known to occur after retrograde infusion into salivary glands (Mason & Chisholm 1975), most likely as a consequence of its insolubility in the tissue. Macrophages present within the parenchyma frequently contained vacuoles with electron-dense material which had a smooth appearance. Similar material was present in vacuoles located in the cytoplasm of the duct cells, and probably constituted remains of the infused medium. The appearance of the vacuoles in both cell types suggests that they were lysosomal, formed by fusion of smaller phagosomes and endocytic structures, and

Fig. 11. Vacuoles (V) of different sizes, are present in the acinar cells (AC). Electron-dense material with a smooth appearance is present in the periphery of one vacuole (arrowheads). Widening of intercellular spaces (arrows) is apparent. Part of a large space surrounded by macrophages (M) is seen. Electron micrograph, 4 days after heavy parenchymal filling, $\times 3,200$.

Fig. 12. Numerous cells containing heterogeneous granules (arrows) are present at the junction between the acini (AC) and intercalated duct (ICD). Electron micrograph, 20 h after heavy parenchymal filling, $\times 3,100$.



probably constituted sites for breakdown of the infused medium. The granular structure of the content seen in some of the vacuoles could be due to the presence of cellular proteins adhering to the contrast medium.

The macrophage-enclosed spaces present in the connective tissue also contained apparent contrast medium, presumably accumulated in the interstitium following parenchymal cell damage. Thus, they may also constitute sites for phagocytosis and breakdown of the infused medium and cellular debris. In addition, these macrophage-enclosed spaces could represent a granulomatous reaction developing due to retention of the lipid-based medium (Mandel & Baurmash 1962, Lilly et al. 1968). Their smaller size and different cellular makeup may reflect a modified response to a smaller amount of the contrast medium than that probably contained in the large cyst-like spaces present in the intralobular connective tissue. Such cyst-like structures have previously been seen in salivary glands after retrograde infusion of glyceryl esters of poppyseed oil (Epsteen & Bendix 1954, Epsteen 1956) and were thought to represent granulomas, formed as a result of a foreign-body reaction to the medium. Our results indicate that infusion of ethyl esters of this compound, the base in Lipiodol UF, can result in a similar reaction. These types of changes are probably partly responsible for the different patterns of recovery observed after administration of the water- and lipid-

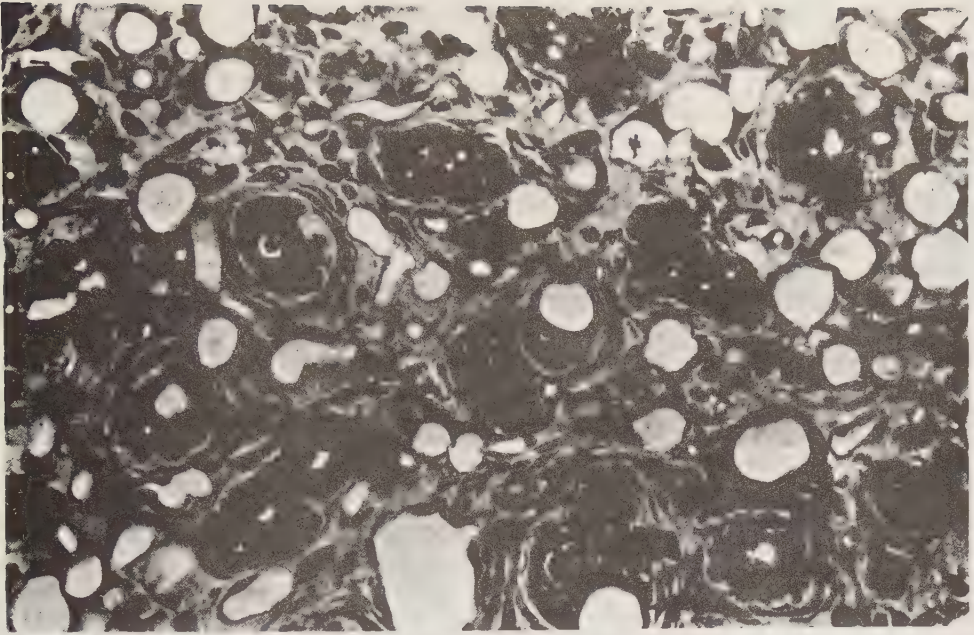
based contrast media, as well as after different degrees of filling with the lipid-soluble medium.

Atrophy of the parenchymal cells and proliferation of the connective tissue were much more pronounced after infusion of the lipid-soluble medium, which could be explained by the more severe alterations. The marked reduction in cell size probably accounts for the displacement and folding of the basement membrane observed in some portions of the parenchyma (Vracko 1978). This has previously been observed in salivary glands after x-irradiation (Sholley et al. 1974), and in streptozotocin-induced diabetes (Weiss & Hand 1981).

The differences in the reactions following infusion of the two media are most probably due to the much higher intraluminal pressure induced during infusion of the lipid-based compound (Qwarnstrom et al. 1981). In addition, secondary alterations caused by parenchymal cell damage and accumulation of cellular debris, as well as changes induced by the presence of the contrast medium, are probably also significant contributing factors. Further, the high pressure developing in the luminal system and the retention of the lipid-based compound may cause alterations or temporary obstruction of the blood flow (Lubarsky & Nicoll 1968). Since intracellular transport and exocytosis of secretory proteins are highly energy-dependent (Jamieson & Palade 1971), the resulting hypoxia would

Fig. 13. Rounded spaces (arrows) surrounded by macrophages are present in a prominent connective-tissue stroma. Atrophy of the parenchymal tissue is apparent. Few acini are seen and some ducts contain vacuoles partially surrounded by macrophages (arrowheads). Light micrograph, 7 days after heavy parenchymal filling, 2.5 μ m, hematoxylin-eosin stain, $\times 320$.

Fig. 14. The intercellular spaces between atrophic parenchymal cells are widened (arrows) and displacement and folding of the basement membrane (BM) is seen. The cells contain numerous vacuoles (V) in their cytoplasm. Lumen (L). Electron micrograph, 7 days after heavy parenchymal filling, $\times 4,800$.



most certainly have a deleterious effect on the secretory process and, thus, induce changes in the parenchymal tissue (Morawa & Han 1974). The more severe alterations occurring after infusion of the lipid-based compound may explain the presence of numerous granular cells at the acinar-intercalated duct junction (Qwarnstrom & Hand 1983). Although the function of these cells is unknown, both their location and increase in number after severe parenchymal damage suggest that they constitute a population of cells differentiating to acini and intercalated duct cells.

Thus, it is clear that either degree of filling with the lipid-soluble radiographic contrast medium results in more severe alterations in the glandular tissue than those caused by infusion of the water-based compound. During the considerably longer recovery period, particularly following heavy parenchymal filling of the gland, additional profound changes may occur in the tissue.

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Saliva Secretion from the Rat Submandibular Gland after Retrograde
Infusion of Radiographic Contrast Media

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ABSTRACT

Rat submandibular saliva was collected at various times after retrograde infusion of water-soluble (Renografin 60%) or lipid-soluble (Lipiodol UF) radiographic contrast medium. Significant alterations in flow rate occurred following heavy parenchymal filling with both types of contrast media. Occasional changes in protein concentration, lactoperoxidase activity and K^+ levels were also noted. These changes were relatively mild and transient, and gland function had generally returned to normal by 1 week after infusion. No alterations in glandular function were detected after infusion of contrast medium to a degree (ductal filling) comparable to that usually employed in clinical sialography.

INTRODUCTION

Sialography is a radiographic examination of the salivary glands that comprises filling of the luminal system with radiographic contrast medium. The examination is used to diagnose various pathological alterations affecting the glands (Mason and Chisholm, 1975; Blair, 1976; Lowman and Cheng, 1976). Light and electron microscopic studies, using the rat submandibular gland as a model, revealed that retrograde infusion causes a number of alterations in the glandular tissue. These consist primarily of dilation of the luminal system, most pronounced immediately after infusion (Qwarnstrom and Hand, 1982 a,b) and a transient inflammatory reaction, peaking at 20 hr (Qwarnstrom et al., 1983; Qwarnstrom and Hand, 1983). The induced alterations may lead to atrophy of the parenchymal tissue, particularly evident one week following the procedure. The severity of the induced changes are dependent on both the medium used and the degree of filling of the gland.

Several pathological conditions result in alterations of the composition of saliva (Mandel, 1980) and analysis of stimulated saliva is often done to evaluate clinically the function of the salivary glands. The present investigation was carried out to determine whether the morphological alterations induced by retrograde infusion can be correlated with alterations in gland function.

MATERIAL AND METHODS

Thirty-two Sprague-Dawley rats (250-400 g each) were used. General anesthesia was achieved by intraperitoneal injection of sodium pentobarbital (45 mg/kg b.w.). After dilation of the duct orifice, the main excretory duct of the left submandibular gland was cannulated intraorally (Omnell and Qwarnstrom, 1983) with polyethylene tubing⁺ pulled out to a tip diameter of 0.5 mm and connected to an infusion pump.[§] The tubing and the syringe attached to the pump were filled with water-soluble[¶] (iodine content 292 mg/ml; viscosity 4 mPa·s at 36°C) or lipid-soluble[#] radiographic contrast medium (iodine content 480 mg/ml; viscosity 25 mPa·s at 36°C). During continuous infusion at a constant rate of 0.008 ml/min the developing pressure was recorded using a monitoring system as previously described (Qwarnstrom and Hand, 1982 b). The glands were subjected to either ductal and slight parenchymal filling or were overfilled (heavy parenchymal filling) as determined by the development of the intraglandular pressure (stages A-B and C, respectively, in Qwarnstrom et al., 1981). The infusion times for the two stages of filling were about 2 and 3.5 minutes using the water-soluble medium, and the maximum pressure reached in both cases was 35-40 mm Hg. Ductal and heavy parenchymal filling were reached after 1.5 and 3 minutes, respectively, using the lipid-based medium, with corresponding maximum pressures of 100 and 150 mm Hg (Qwarnstrom and Hand, 1982 a,b).

⁺ PE 7410, Clay Adams, Parsippany, NJ 07054

[§] Syringe pump Model 314 A, Sage Instruments, Cambridge, MA 02139

[¶] Renografin 60%, Squibb, Princeton, NJ 08540

[#] Lipiodol UF, Guerbet, Aulnay-sous-Bois, France

At different times after infusion (immediately, 20 hr, 1 week and in some cases 2 weeks) both right and left submandibular glands were cannulated intra-orally with a polyethylene tubing open to the air. Secretion was stimulated by pilocarpine-HCl^{**} (2.5 mg/kg b.w., s.c.). The initial drop was discarded, and subsequent flow was collected for 30 min from each gland into separate pre-weighed plastic tubes kept on ice. A total of 51 saliva collections were made from the 32 animals. Following the collection period, the tubes were re-weighed and the saliva was quickly frozen on dry ice and stored at -20°C until analyzed. Immediately prior to analysis, the samples were thawed in an ice bath and diluted with ethylene diaminetetraacetate (EDTA) to a final concentration of 1 mM to prevent formation of a calcium-protein precipitate (Dawes, 1981). For each analysis, all samples were assayed at least in duplicate to minimize error. The saliva samples were analyzed for the following parameters: flow rate, the concentrations of total protein, lactoperoxidase, Na⁺ and K⁺. In addition, the albumin concentration of each sample was measured.

Flow rate was determined gravimetrically assuming a specific gravity of saliva equal to 1 and expressed as $\mu\text{l}/\text{min} \cdot \text{gland}$. Total protein was determined by absorbance at 215 nm (Arneberg, 1971) using bovine serum albumin as a standard. Na⁺ and K⁺ concentrations were measured by atomic absorption spectrometry.⁺⁺ Lactoperoxidase activity was determined by measuring the oxidation of iodine in the presence of hydrogen peroxide at 350 nm (Morrison, 1970). Albumin was

^{**} Sigma Chemical Co., St. Louis, MO 63178

⁺⁺ Model 306, Perkin-Elmer, Norwalk, CT 06856

determined using an enzyme-linked immunosorbent assay (ELISA) similar to that described previously (Baum et al., 1982). Rat serum albumin^{\$\$} was used as a standard. Determination of the albumin content of rat serum by this assay yielded results of 2.6-4.0% and concentrations as low as 1.0 ng/ml could readily be measured.

The parameter values obtained from saliva of the infused gland of each animal were compared with respective values from the contralateral gland that served as a control. The means of the calculated ratios (left gland/right gland) were compared with corresponding means of ratios from rats where neither gland had been infused with contrast medium (from the series by Bodner et al., 1983).

^{\$\$} Miles, Elkhart, IN 46515

RESULTS

The mean values for the measured parameters from right and left submandibular glands of control animals together with the means of the ratios (left gland/right gland) from each animal are shown in Table 1. The coefficient of variation for the mean ratio from individual animals is consistently less than that for either the left or right gland taken separately. This is consistent with the observation by Bodner et al. (1983), that the contralateral gland is a useful control.

As shown in Tables 2 and 3, for most parameters the values obtained for the infused glands are comparable to those of the contralateral gland, i.e., ratios ≈ 1 . Ductal filling did not affect the gland function significantly for any of the criteria followed, whereas some alterations occurred following heavy parenchymal filling with either medium.

The flow rate was most notably affected by heavy parenchymal filling. A significant reduction occurred at 20 hr after infusion of either medium (Tables 2 and 3). The flow rate was also reduced immediately after retrograde filling with lipid-soluble medium, but this was not statistically significant. By one week after infusion, the flow rate had returned to near control levels.

A few significant alterations in the other parameters were observed at various times after infusion, but no particular pattern to these changes could be discerned. The protein concentration of the saliva was increased immediately following heavy parenchymal filling with water-soluble medium (Table 2). By 20 hr, however, the protein concentration had returned to control levels. An increase in protein concentration occurred at this time in saliva from glands subjected to heavy parenchymal filling with lipid-soluble medium, but was not

significant ($0.05 < p < 0.1$). A high lactoperoxidase activity was noted in saliva from some experimental glands 1 week after heavy parenchymal filling with either medium. However, the mean ratio of the group was not significantly different from controls ($0.05 < p < 0.1$) (Tables 2 and 3). Levels of Na^+ and K^+ were essentially unchanged, except for a small but significant elevation in K^+ 20 hr after infusion of lipid-soluble medium (Table 3).

Control saliva contained only trace amounts of serum albumin, as determined by the sensitive ELISA method employed. No significant changes in albumin concentration were observed in saliva from any of the experimental glands.

DISCUSSION

Alterations in the measured parameters were found only in saliva from glands subjected to overfilling with either medium. Moderate dilation of the luminal system such as occurs following ductal filling (Qwarnstrom and Hand, 1982 a,b) apparently does not affect secretion of the gland to a significant degree. More pronounced alterations, caused by prolonged infusion, may be correlated with functional changes.

The reduction in flow rate could be due to a number of factors, most of which are directly or indirectly related to the increased pressure induced by infusion. Secretory dysfunction may be a result of damage to the acinar and duct cells. Morphological changes were prominent in the acini and ducts at 20-30 hr after overfilling (Qwarnstrom et al., 1983; Qwarnstrom and Hand, 1983) - the time when the greatest reduction in saliva flow was observed in the present study. Changes likely to influence saliva production include: dilated lumina; scalloping of the luminal membrane and compression of the duct cells; fusion of secretory granules and formation of vacuoles in the acinar cells. Leakage through altered junctional complexes (Qwarnstrom and Hand, 1982 a,b) may also account for part of the reduction in saliva volume. Reduced salivary flow has previously been reported after release of ductal ligation (Martinez et al., 1982), and after transient increases in intraluminal pressure (Siegel, 1976; Dawes et al., 1980). Using the lipid-based medium, the effect on the flow rate was more pronounced and also occurred at an earlier time after infusion, which most likely reflects the severity of the morphological alterations induced during infusion of this medium (Qwarnstrom and Hand, 1982 b). This is probably due to the considerably higher pressure developing during infusion (Qwarnstrom et al., 1981), resulting from the higher

viscosity and the insolubility of this medium. In addition to various degrees of damage induced by the infusion pressure, retention of the lipid-soluble medium in the luminal system (Mason and Chisholm, 1975) may result in blockage of parts of the gland containing excess contrast medium, with a consequent reduction in flow. The development of an inflammatory reaction in the gland may also result in reduced secretion. The peak of the inflammatory response after heavy parenchymal filling with either medium occurs at 20 hr (Qwarnstrom et al., 1983; Qwarnstrom and Hand, 1983), coinciding with the most pronounced reductions in flow. In humans, reduced flow is commonly associated with acute and chronic inflammatory reactions of the salivary glands (Mandel, 1980). The significant increase in K^+ levels noted at 20 hr following heavy parenchymal filling with the lipid-soluble medium, may reflect the extensive structural alterations in the various portions of the duct system noted at this time (Qwarnstrom and Hand, 1983), including the presence of large cytoplasmic vacuoles. Interestingly, the non-inflammatory sialadenoses are usually characterized by reduced flow rates and elevated K^+ levels (Rauch and Gorlin, 1970), similar to the changes we observed in these two parameters.

The increase in protein concentration noted at early times after overfilling with either medium may also be due to cellular damage induced during infusion. The alterations do not appear to be due to leakage of serum proteins across the glandular epithelium, since no significant changes were seen in saliva albumin concentration.

Generally, the alterations in the measured parameters were few and not significant which was unexpected considering the severe morphological alterations observed, both directly following infusion (Qwarnstrom and Hand, 1982 a,b) and during recovery (Qwarnstrom et al.,

1983; Qwarnstrom and Hand, 1983). Due to the uneven distribution of contrast medium, considerable variability occurs in the degree and extent of these alterations, and some parts of most glands presumably remain relatively intact. It is possible that these less-altered regions can provide for a normal secretion, or that some compensatory mechanism is involved and responsible for the continuous function of the gland.

In conclusion, it is apparent that although retrograde infusion of radiographic contrast media may cause some transient functional disturbances to the rat submandibular gland, a rapid recovery occurs. At present it is not possible to make a direct correlation between the data obtained in this study and the clinical situation. For example, in the unanesthetized patient, the activity of myoepithelial cells will be maintained, resulting in a reduced distensibility of the acini and intercalated ducts (Emmelin et al., 1977). Further, retrograde infusion into a gland that is already compromised by a pathological condition could potentially result in more severe alterations. The results from the present study provide a basis for the types of alterations that can occur, although the sequence and severity of the changes induced during clinical sialography may be quite different. In addition, this study demonstrates that the degree of filling of the gland is of importance and the likelihood of inducing functional disturbances is considerably reduced by using ductal filling.

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TABLE 1

Comparison of Left and Right Submandibular Gland Secretion
from Control Animals*

	Left Gland	Right Gland	Ratio, Left/Right
Flow rate, $\mu\text{l}/\text{min}$ gland	$15.39 \pm 8.19^+ (0.53)^{\S}$	$16.58 \pm 6.51^+ (0.39)^{\S}$	$1.04 \pm 0.27^+ (0.26)^{\parallel}$
Protein, mg/ml	$4.18 \pm 1.67 (0.40)$	$4.02 \pm 1.75 (0.44)$	$1.07 \pm 0.13 (0.12)$
Lactoperoxidase, U/mg protein	$0.71 \pm 0.26 (0.37)$	$0.66 \pm 0.24 (0.36)$	$1.19 \pm 0.36 (0.30)$
Na^+ , mM	$12.73 \pm 5.64 (0.44)$	$12.13 \pm 6.07 (0.50)$	$1.11 \pm 0.30 (0.30)$
K^+ , mM	$39.54 \pm 9.69 (0.25)$	$37.93 \pm 7.49 (0.20)$	$1.04 \pm 0.12 (0.11)$

* Eight animals selected at random from the series reported by Bodner et al. (1983).

+ Mean $\pm$ S.D.

$\S$ Numbers in parentheses after left and right gland values are the coefficients of variation for each parameter studied.

$\parallel$ Coefficient of variation for left gland/right gland ratios for individual animals.

TABLE 2

Comparison of Left and Right Rat Submandibular Gland Secretion
after Retrograde Infusion of Water-Soluble Contrast Medium *

	Time after Ductal Filling ⁺		Time after Heavy Parenchymal Filling ⁺	
	Immediately	20 hr	Immediately	20 hr
Flow rate	1.03 ± 0.23	1.01 ± 0.01	1.20 ± 0.11	0.73 ± 0.20 [§]
Protein	1.40 ± 0.61	1.06 ± 0.51	1.49 ± 0.25 [§]	0.83 ± 0.21
Lactoperoxidase	0.89 ± 0.37	0.69 ± 0.31	0.87 ± 0.12	1.07 ± 0.68
Na ⁺	1.56 ± 0.48	0.91 ± 0.07	1.18 ± 0.34	1.33 ± 0.24
K ⁺	0.98 ± 0.22	1.01 ± 0.02	1.12 ± 0.15	0.98 ± 0.04
				0.97 ± 0.09

* Mean ratios ± S.D. of parameter values (Left gland/Right gland) for 2-8 animals at each time point. Left gland had been infused with contrast medium.

⁺ For statistical evaluation, ratios obtained from experimental animals were compared with those from controls shown in Table 1 by an unpaired t test and by a nonparametric test (Mann-Whitney).

[§] p < 0.05.

TABLE 3

Comparison of Left and Right Rat Submandibular Gland Secretion after Retrograde Infusion of Lipid-Soluble Contrast Medium^{*}

	Time after Ductal Filling ⁺			Time after Heavy Parenchymal Filling ⁺		
	Immediately	20 hr	1 week	Immediately	20 hr	1 week 2 weeks
Flow rate	1.27 ± 0.49	1.11 ± 0.05	1.05 ± 0.09	0.59 ± 0.46	0.41 ± 0.35 [§]	0.82 ± 0.32 1.01 ± 0.16
Protein	0.99 ± 0.14	0.90 ± 0.13	1.04 ± 0.16	1.05 ± 0.08	1.50 ± 0.50	1.07 ± 0.08 0.91 ± 0.13
Lactoperoxidase	1.30 ± 0.58	1.13 ± 0.23	0.91 ± 0.09	1.00 ± 0.43	1.41 ± 0.33	1.38 ± 0.61 1.08 ± 0.16
Na ⁺	1.10 ± 0.39	0.94 ± 0.01	0.99 ± 0.20	1.41 ± 0.37	0.92 ± 0.15	0.96 ± 0.08 0.99 ± 0.05
K ⁺	0.94 ± 0.12	0.94 ± 0.07	1.00 ± 0.08	1.10 ± 0.07	1.26 ± 0.09 [§]	1.12 ± 0.19 1.06 ± 0.06

* Mean ratios ± S.D. of parameter values (Left gland/Right gland) for 2-7 animals at each time point. Left gland had been infused with contrast medium.

⁺ For statistical evaluation, ratios obtained from experimental animals were compared with those from controls shown in Table 1 by an unpaired t test and by a nonparametric test (Mann-Whitney).

[§] p < 0.05.

